

EFFECTS OF IONIC PERTURBATIONS AND METAL ION  
COMPETITION ON THE BINDING OF A MODEL DRUG TO DNA

By

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## DEDICATION

To Dearest Joannie  
who, more than any other,  
deserves credit for any positive  
attributes I may possess.

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## TABLE OF CONTENTS

|                                                                                            | <u>Page</u> |
|--------------------------------------------------------------------------------------------|-------------|
| ACKNOWLEDGEMENTS.....                                                                      | iii         |
| LIST OF FIGURES.....                                                                       | vi          |
| LIST OF TABLES.....                                                                        | x           |
| ABSTRACT.....                                                                              | xiv         |
| CHAPTER I - INTRODUCTION.....                                                              | 1           |
| Binding of Small Molecules to Biopolymers.....                                             | 2           |
| Thermodynamics of Binding Reactions.....                                                   | 34          |
| Rationale for the Selection of the Model<br>System and Experimental Design.....            | 42          |
| CHAPTER II - EXPERIMENTAL.....                                                             | 47          |
| Materials and Procedures.....                                                              | 47          |
| Calculations.....                                                                          | 55          |
| CHAPTER III - RESULTS AND DISCUSSION.....                                                  | 62          |
| General Spectral and Titration Characteristics..                                           | 62          |
| Apparent Association Constants for Surface<br>and Intercalative Binding.....               | 84          |
| Association Constants Based on Activities --<br>Extended Debye-Hückel Considerations.....  | 97          |
| Evaluation of a Simple Competitive Binding<br>Model.....                                   | 102         |
| Thermodynamics of the Binding of 3-amino-<br>acridinium and 7-aminoquinolinium to DNA..... | 105         |
| APPENDICES                                                                                 |             |
| APPENDIX I - FIGURES.....                                                                  | 120         |



TABLE OF CONTENTS (Continued)

|                                       | <u>Page</u> |
|---------------------------------------|-------------|
| APPENDIX II - TABLES.....             | 187         |
| APPENDIX III - COMPUTER PROGRAMS..... | 281         |
| REFERENCES.....                       | 295         |
| BIOGRAPHICAL SKETCH.....              | 301         |

# LIST OF FIGURES

| <u>Figure</u> |                                                                                                                                                                              | <u>Page</u> |
|---------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|
| 1             | The structures of selected substituted acridines and related compounds (Structures I through XVI)                                                                            | 122         |
| 2             | General form of Scatchard plots: $R/C$ vs. $R$ for one and two classes of binding sites                                                                                      | 128         |
| 3             | Absorptiometric titration of 3-amino-acridinium cation with DNA, cesium salt, pH 5.90, 25.0°C. Background electrolyte: $C_5H_2PO_4$ , initial concentration, 0.15M           | 130         |
| 4             | Absorptiometric titration of 3-amino-acridinium cation with DNA, cesium salt, pH 5.90, 25.0°C. Background electrolyte: $C_5H_2PO_4$ , initial concentration, 0.0025M         | 132         |
| 5             | Absorptiometric titration of 3-amino-acridinium cation with DNA, magnesium salt, pH 5.90, 25.0°C. Background electrolyte: $Mg(O_2CCH_3)_2$ , initial concentration, 0.010N   | 134         |
| 6             | Absorptiometric titration of 3-amino-acridinium cation with DNA, magnesium salt, pH 5.90, 25.0°C. Background electrolyte: $Mg(O_2CCH_3)_2$ , initial concentration, 0.00063N | 136         |
| 7             | $\log([BHP]/[BH])$ vs. $\log([P_t/3] - [BHP])$ with 0.0025M $C_5H_2PO_4$ and $6.3 \times 10^{-4}N$ $Mg(O_2CCH_3)_2$ , as supporting electrolytes, pH 5.90, 25.0°C            | 138         |
| 8             | $\log([BHP]/[BH])$ vs. $\log([P_t/3] - [BHP])$ with 0.15M $C_5H_2PO_4$ , 0.010M $C_5H_2PO_4$ , and 0.010N $Mg(O_2CCH_3)_2$ as supporting electrolytes, pH 5.90, 25.0°C       | 140         |
| 9             | $\log([BHP]/[BH])$ vs. $\log([P_t/2] - 2[BHP])$ with 0.0025M $C_5H_2PO_4$ and $6.3 \times 10^{-4}N$ $Mg(O_2CCH_3)_2$ as supporting electrolytes, pH 5.90, 25.0°C             | 142         |

# LIST OF FIGURES (Continued)

| Figure |                                                                                                                                                                                                                                                                                                                                          | Page |
|--------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| 10     | Log([BHP]/[BH]) vs. log([P <sub>t</sub> /2]-2[BHP]) with 0.15M CsH <sub>2</sub> PO <sub>4</sub> , 0.01M CsH <sub>2</sub> PO <sub>4</sub> , and 0.010N Mg(O <sub>2</sub> CCH <sub>3</sub> ) <sub>2</sub> as supporting electrolytes, pH 5.90, 25.0°C                                                                                      | 144  |
| 11     | Log of the apparent association constant, K <sub>s</sub> , for the surface binding of 3-amino-acridinium cation to DNA vs. the square root of the ionic strength, I <sup>1/2</sup> . NaH <sub>2</sub> PO <sub>4</sub> and LiH <sub>2</sub> PO <sub>4</sub> as supporting electrolytes, pH 5.90, 25.0°C                                   | 146  |
| 12     | Log of the apparent association constant, K <sub>s</sub> , for the surface binding of 3-amino-acridinium cation to DNA vs. the square root of the ionic strength, I <sup>1/2</sup> . KH <sub>2</sub> PO <sub>4</sub> and KO <sub>2</sub> CCH <sub>3</sub> as supporting electrolytes, pH 5.90, 25.0°C                                    | 148  |
| 13     | Log of the apparent association constant, K <sub>s</sub> , for the surface binding of 3-amino-acridinium cation to DNA vs. the square root of the ionic strength, I <sup>1/2</sup> . KH <sub>2</sub> PO <sub>4</sub> and RbH <sub>2</sub> PO <sub>4</sub> as supporting electrolytes, pH 5.90, 25.0°C                                    | 150  |
| 14     | Log of the apparent association constant, K <sub>s</sub> , for the surface binding of 3-amino-acridinium cation to DNA vs. the square root of the ionic strength, I <sup>1/2</sup> . CsH <sub>2</sub> PO <sub>4</sub> and (CH <sub>3</sub> ) <sub>4</sub> NH <sub>2</sub> PO <sub>4</sub> as supporting electrolytes, pH 5.90, 25.0°C    | 152  |
| 15     | Log of the apparent association constant, K <sub>s</sub> , for the surface binding of 3-amino-acridinium cation to DNA vs. the square root of the ionic strength, I <sup>1/2</sup> . Mg(O <sub>2</sub> CCH <sub>3</sub> ) <sub>2</sub> and Ca(O <sub>2</sub> CCH <sub>3</sub> ) <sub>2</sub> as supporting electrolytes, pH 5.90, 25.0°C | 154  |
| 16     | Log of the apparent association constant, K <sub>s</sub> , for the surface binding of 3-amino-acridinium cation to DNA vs. the square root of the ionic strength, I <sup>1/2</sup> . Sr(O <sub>2</sub> CCH <sub>3</sub> ) <sub>2</sub> and Ba(O <sub>2</sub> CCH <sub>3</sub> ) <sub>2</sub> as supporting electrolytes, pH 5.90, 25.0°C | 156  |

# LIST OF FIGURES (Continued)

| <u>Figure</u> |                                                                                                                                                                                                                                                                            | <u>Page</u> |
|---------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|
| 17            | Log of the apparent association constant, $K_I$ , for the intercalative binding of 3-aminoacridinium cation to DNA vs. the square root of the ionic strength, $I^{1/2}$ . $LiH_2PO_4$ and $NaH_2PO_4$ as supporting electrolytes, pH 5.90, 25.0°C                          | 158         |
| 18            | Log of the apparent association constant, $K_I$ , for the intercalative binding of 3-aminoacridinium cation to DNA vs. the square root of the ionic strength, $I^{1/2}$ . $KH_2PO_4$ and $KO_2CCH_3$ as supporting electrolytes, pH 5.90, 25.0°C                           | 160         |
| 19            | Log of the apparent association constant, $K_I$ , for the intercalative binding of 3-aminoacridinium cation to DNA vs. the square root of the ionic strength, $I^{1/2}$ . $KH_2PO_4$ and $RbH_2PO_4$ as supporting electrolytes, pH 5.90, 25.0°C                           | 162         |
| 20            | Log of the apparent association constant, $K_I$ , for the intercalative binding of 3-aminoacridinium cation to DNA vs. the square root of the ionic strength, $I^{1/2}$ . $CsH_2PO_4$ and $(CH_3)_4NH_2PO_4$ as supporting electrolytes, pH 5.90, 25.0°C                   | 164         |
| 21            | Log of the corrected association constant, $K'_S$ , for the surface binding of 3-aminoacridinium cation to DNA vs. the square root of the activity of solution, $I^{1/2}$ . $LiH_2PO_4$ , $NaH_2PO_4$ , and $KH_2PO_4$ as supporting electrolytes, pH 5.90, 25.0°C         | 166         |
| 22            | Log of the corrected association constant, $K'_S$ , for the surface binding of 3-aminoacridinium cation to DNA vs. the square root of the activity of solution, $I^{1/2}$ . $RbH_2PO_4$ , $CsH_2PO_4$ , and $(CH_3)_4NH_2PO_4$ as supporting electrolytes, pH 5.90, 25.0°C | 168         |
| 23            | Log of the corrected association constant, $K'_S$ , for the surface binding of 3-aminoacridinium cation to DNA vs. the square root of the activity of solution, $I^{1/2}$ . $Mg(O_2CCH_3)_2$ and $Ca(O_2CCH_3)_2$ as supporting electrolytes, pH 5.90, 25.0°C              | 170         |

# LIST OF FIGURES (Continued)

| <u>Figure</u> |                                                                                                                                                                                                                                                               | <u>Page</u> |
|---------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|
| 24            | Log of the corrected association constant, $K'_S$ , for the surface binding of 3-aminoacridinium cation to DNA vs. the square root of the activity of solution, $I^{1/2}$ . $Sr(O_2CCH_3)_2$ and $Ba(O_2CCH_3)_2$ as supporting electrolytes, pH 5.90, 25.0°C | 172         |
| 25            | Absorptiometric titration of 7-aminoquinolinium cation with DNA, potassium salt, pH 5.90, 25.0°C. Background electrolyte: $KH_2PO_4$ , initial concentration, 0.0050M                                                                                         | 174         |
| 26            | $\log([AHP]/[AH])$ vs. $\log([P_t/3]-[AHP])$ for the binding of 7-aminoquinolinium cation to DNA at 15.0°C, 25.0°C, and 35.0°C, pH 5.90, 0.010M $KH_2PO_4$ as supporting electrolyte                                                                          | 176         |
| 27            | $\log([AHP]/[AH])$ vs. $\log([P_t/2]-2[AHP])$ for the binding of 7-aminoquinolinium cation to DNA at 15.0°C, 25.0°C, and 35.0°C, pH 5.90, 0.010M $KH_2PO_4$ as supporting electrolyte                                                                         | 178         |
| 28            | $\log K'_S$ vs. $1/T(^{\circ}K)$ for the reaction between 3-aminoacridinium cation and DNA at 15.0°C, 25.0°C, and 35.0°C, pH 5.90, 0.010M $KH_2PO_4$ as supporting electrolyte                                                                                | 180         |
| 29            | $\log K'_S$ vs. $1/T(^{\circ}K)$ for the reaction between 7-aminoquinolinium cation and DNA at 15.0°C, 25.0°C, and 35.0°C, pH 5.90, 0.010M $KH_2PO_4$ as supporting electrolyte                                                                               | 182         |
| 30            | $\log K'_S$ vs. $1/T(^{\circ}K)$ for the reaction between 3-aminoacridinium cation and DNA at 15.0°C, 25.0°C, and 35.0°C, pH 5.90, 0.010M $KH_2PO_4$ as supporting electrolyte                                                                                | 184         |
| 31            | $\log K'_S$ vs. $1/T(^{\circ}K)$ for the reaction between 7-aminoquinolinium cation and DNA at 15.0°C, 25.0°C, and 35.0°C, pH 5.90, 0.010M $KH_2PO_4$ as supporting electrolyte                                                                               | 186         |

# LIST OF TABLES

| <u>Table</u> |                                                                                                                                                               | <u>Page</u> |
|--------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|
| 1            | Molar absorptivities of the 3-amino-acridinium-DNA complex and the 7-amino-quinolinium-DNA complex in various supporting electrolytes                         | 188         |
| 2            | Absorptiometric titration of 3-amino-acridinium cation with DNA, lithium salt. Lithium dihydrogen phosphate as supporting electrolyte                         | 189         |
| 3            | Absorptiometric titration of 3-amino-acridinium cation with DNA, sodium salt. Sodium dihydrogen phosphate as supporting electrolyte                           | 194         |
| 4            | Absorptiometric titration of 3-amino-acridinium cation with DNA, potassium salt. Potassium dihydrogen phosphate as supporting electrolyte                     | 198         |
| 5            | Absorptiometric titration of 3-amino-acridinium cation with DNA, potassium salt. Potassium acetate as supporting electrolyte                                  | 202         |
| 6            | Absorptiometric titration of 3-amino-acridinium cation with DNA, rubidium salt. Rubidium dihydrogen phosphate as supporting electrolyte                       | 207         |
| 7            | Absorptiometric titration of 3-amino-acridinium cation with DNA, cesium salt. Cesium dihydrogen phosphate as supporting electrolyte                           | 212         |
| 8            | Absorptiometric titration of 3-amino-acridinium cation with DNA, tetramethylammonium salt. Tetramethylammonium dihydrogen phosphate as supporting electrolyte | 216         |

# LIST OF TABLES (Continued)

| <u>Table</u> |                                                                                                                                                                                            | <u>Page</u> |
|--------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|
| 9            | Absorptiometric titration of 3-amino-acridinium cation with DNA, magnesium salt. Magnesium acetate as supporting electrolyte                                                               | 222         |
| 10           | Absorptiometric titration of 3-amino-acridinium cation with DNA, calcium salt. Calcium acetate as supporting electrolyte                                                                   | 226         |
| 11           | Absorptiometric titration of 3-amino-acridinium cation with DNA, strontium salt. Strontium acetate as supporting electrolyte                                                               | 230         |
| 12           | Absorptiometric titration of 3-amino-acridinium cation with DNA, barium salt. Barium acetate as supporting electrolyte                                                                     | 234         |
| 13           | Absorptiometric titration of 3-amino-acridinium cation with DNA, potassium salt, at 15°C                                                                                                   | 239         |
| 14           | Absorptiometric titration of 3-amino-acridinium cation with DNA, potassium salt, at 35°C                                                                                                   | 241         |
| 15           | Absorptiometric titration of 7-amino-quinolinium cation with DNA, potassium salt, at 15°C                                                                                                  | 243         |
| 16           | Absorptiometric titration of 7-amino-quinolinium cation with DNA, potassium salt, at 25°C                                                                                                  | 245         |
| 17           | Absorptiometric titration of 7-amino-quinolinium cation with DNA, potassium salt, at 35°C                                                                                                  | 247         |
| 18           | Calculated values of [BH], [BHP], "K <sub>S</sub> ", and related data for the reaction between 3-aminoacridinium cation and DNA in 0.15M CSH <sub>2</sub> PO <sub>4</sub>                  | 249         |
| 19           | Calculated values of [BH], [BHP], K <sub>S</sub> , K <sub>I</sub> , and related data for the reaction between 3-aminoacridinium cation and DNA in 0.0025M CSH <sub>2</sub> PO <sub>4</sub> | 251         |



# LIST OF TABLES (Continued)

| <u>Table</u>                                                                                                                                                                     | <u>Page</u> |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|
| 20     Calculated values of [BH], [BHP], $K_S$ , " $K_I$ ", and related data for the reaction between 3-aminoacridinium cation and DNA in 0.010M $Mg(O_2CCH_3)_2$                | 253         |
| 21     Calculated values of [BH], [BHP], $K_S$ , " $K_I$ ", and related data for the reaction between 3-aminoacridinium cation and DNA in $6.3 \times 10^{-4}N$ $Mg(O_2CCH_3)_2$ | 255         |
| 22     Percent total 3-aminoacridinium bound to DNA in the presence of various electrolytes after addition of excess DNA                                                         | 257         |
| 23     Initial and final slopes of plots of $\log([BHP]/[BH])$ vs. $\log([P_t/m] - q[BHP])$ in various concentrations of $CsH_2PO_4$ and $Mg(O_2CCH_3)_2$                        | 258         |
| 24     Apparent equilibrium association constants for surface, $K_S$ , and intercalative, $K_I$ , binding of 3-aminoacridinium to DNA, $LiH_2PO_4$ as supporting electrolyte     | 259         |
| 25     Apparent equilibrium association constants for surface, $K_S$ , and intercalative, $K_I$ , binding of 3-aminoacridinium to DNA, $NaH_2PO_4$ as supporting electrolyte     | 261         |
| 26     Apparent equilibrium association constants for surface, $K$ , and intercalative, $K$ , binding of 3-aminoacridinium to DNA, $KH_2PO_4$ as supporting electrolyte          | 262         |
| 27     Apparent equilibrium association constants for surface, $K_S$ , and intercalative, $K_I$ , binding of 3-aminoacridinium to DNA, $KO_2CCH_3$ as supporting electrolyte     | 263         |
| 28     Apparent equilibrium association constants for surface, $K_S$ , and intercalative, $K_I$ , binding of 3-aminoacridinium to DNA, $RbH_2PO_4$ as supporting electrolyte     | 265         |
| 29     Apparent equilibrium association constants for surface, $K_S$ , and intercalative, $K_I$ , binding of 3-aminoacridinium to DNA, $CsH_2PO_4$ as supporting electrolyte     | 267         |



# LIST OF TABLES (Continued)

| <u>Table</u> |                                                                                                                                                                                    | <u>Page</u> |
|--------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|
| 30           | Apparent equilibrium association constants for the surface, $K_s$ , and intercalative, $K_I$ , binding of 3-aminoacridinium to DNA, $(CH_3)_4^-NH_2PO_4$ as supporting electrolyte | 269         |
| 31           | Apparent equilibrium association constants, $K_s$ , for surface binding of 3-aminoacridinium to DNA, $Mg(O_2CCH_3)_2$ and $Ca(O_2CCH_3)_2$ as supporting electrolytes              | 271         |
| 32           | Apparent equilibrium association constants, $K$ , for the surface binding of 3-aminoacridinium to DNA, $Sr(O_2CCH_3)_2$ and $Ba(O_2CCH_3)_2$ as supporting electrolytes            | 273         |
| 33           | Logarithms of the apparent surface binding association constants for the binding of 3-aminoacridinium cation to DNA, corrected for ion activities                                  | 275         |
| 34           | Equilibrium association constants for the binding of 3-aminoacridinium to DNA, $K_s''$ , and for the binding of alkali and alkaline earth metal cations to DNA, $K_M$              | 277         |
| 35           | Apparent association constants for the surface, $K_s$ , and intercalative, $K_I$ , binding of 3-aminoacridinium and 7-aminoquinolinium to DNA at 15.0°C, 25.0°C, and 35.0°C        | 279         |
| 36           | Thermodynamic parameters for the surface and intercalative binding reactions between 3-aminoacridinium and 7-aminoquinolinium cations to DNA                                       | 280         |

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EFFECTS OF IONIC PERTURBATIONS AND METAL ION  
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Absorptiometric titrations of a model drug compound, 3-aminoacridinium cation, with salts of calf thymus DNA were carried out at pH 5.90 in a series of solutions in which the supporting electrolyte was the dihydrogen phosphate salt of one of the following: lithium, sodium, potassium, rubidium, cesium, or tetramethylammonium ion. Titrations were also conducted in solutions of the acetate salts of magnesium, calcium, strontium, and barium. The ionic strengths of the solutions were varied between 0.25 and 0.00063(M) for each alkali metal electrolyte and between 0.036 and 0.00010 for the alkaline earth electrolytes. Apparent equilibrium association constants for the surface

binding and for the intercalative binding of 3-amino-acridinium cation to DNA were computed for each concentration of each alkali metal electrolyte solution and are reported. Apparent constants for the surface binding of 3-aminoacridinium in the presence of alkaline earths are also presented. Values for the intercalative binding mode in the presence of group IIA metal cations could not be determined.

The extent of binding of the model drug to DNA was dependent on the nature and concentration of the electrolyte. Essentially complete binding was attainable for all concentrations of all alkali metals while as little as 70% binding was possible in the presence of 0.010N magnesium. Spectral characteristics of the drug-DNA complex were also dependent upon the nature of the background electrolyte and are discussed.

The reaction systems did not behave in accordance with limiting Debye-Hückel relationships over the range of ionic strength investigated. After applying extended Debye-Hückel corrections, the relationships between the surface-binding equilibrium association constants and the ionic strength indicated that specific-ion competition between metal and drug cations, for DNA phosphate sites, was significant. A simple 1:1 competitive model was evaluated.

At ionic strengths less than about 0.010, experimental precision was poor, presumably due to alterations in the conformation of DNA at low ionicities, suggesting that

evaluations of interactions between small molecules and DNA be conducted in solutions of ionic strength in excess of 0.010.

Thermodynamic parameters for surface and intercalative binding of 3-aminoacridinium cation and its benzolog, 7-aminoquinolinium, were evaluated from equilibrium association constants at 15.0°C, 25.0°C, and 35.0°C. The standard enthalpy change,  $\Delta H^\circ$ , for the 3-aminoacridinium binding was less negative than  $\Delta H^\circ$  for the 7-aminoquinolinium process, while the standard entropy change,  $\Delta S^\circ$ , for the former was more positive than for the latter. Thus, the greater degree of disordering resulting from the relaxation of the solvent around the tricyclic 3-aminoacridinium cation as it binds to DNA appears responsible for the more negative standard free energy change for the 3-aminoacridinium binding process. Values of  $\Delta S^\circ$  for the intercalative binding of the two drugs suggest that significant ordering of the polymer helix occurs upon intercalation.

## CHAPTER I

### INTRODUCTION

The interaction of small molecules with DNA and biopolymers, in general, has been the subject of intense investigation during the past several decades. Interest in this area has been due, in large part, to the early realization that such interactions may result in mutagenesis (1) and carcinogenesis (2), especially when the small molecules are cationic aromatics such as substituted acridines. Numerous publications deal with various aspects of the interactions of cationic aromatics with DNA, some of which will be briefly reviewed here. The specific systems to be considered in this work include 3-aminoacridine(I).<sup>1</sup> Hence, a summary of representative and pertinent research involving aminoacridines is included. Moreover, we have investigated the effects of metal ions on the interactions of 3-aminoacridine with DNA and, therefore, shall review the past research efforts in this area. It is emphasized that this review is, by no means,

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<sup>1</sup>Roman numerals in parentheses after chemical names refer to their listings in Figure 1 of appendix I.

exhaustive, but rather, is intended to provide perspective and an introduction to the work that is included in this report.

### Binding of Small Molecules to Biopolymers

Probably the first acknowledgement of a significant interaction between a member of the acridine chemical family and a biological system was reported in 1913 by Ehrlich and Benda (3) when they reported their observation on the antibacterial activity of 3,6-diamino-10-methyl-acridinium chloride(II) against Trypanosonoma brucei. Subsequently, evidence accumulated that the antibacterial activity of substituted aminoacridines is dependent on their capacity to compete with hydronium ion for a vital anionic site in a bacterium (4-6, 7: Ch. 2) and that antibacterial activity and mammalian toxicity were not directly related (8,9). The antibacterial action of proflavine(III) is reported to be due to its ability to inhibit DNA-dependent RNA synthesis (10) which suggests that DNA may contain the anionic site. However, it is inadvisable, at this time, to make a direct correlation since proflavine also binds strongly to a variety of other cellular structures, any one of which may contain the vital anionic site (11).

Numerous amino-substituted acridines are known to be carcinogenic while few simple acridines are cancer inducing (12) which may be due to the former's greater ability to

intercalate into DNA (13). It is worthy of note that benzacridines(IV) and dibenzacridines(V), first extensively studied in 1935, are nearly all highly carcinogenic (2). Two possible modes of action have been proposed. The benzacridine may be metabolically activated to a cationic form which then reacts as an electrophile toward nucleophilic centers of DNA, RNA, and protein (14), or the unmetabolized species may interact physically with the DNA (15), perhaps by intercalating into the double stranded helix.

Mutagenesis, presumably arising from the intercalation of acridines with nucleic acids, was first reported by DeMars in 1953 (1) in relation to his work on proflavine and T2 bacteriophage (Escherichia coli). The mutagenic activity of proflavine does not involve its integral incorporation into the newly synthesized polynucleotide chain but rather, results from the acridine's capacity to cause deletions and insertions of nucleotides into the DNA polynucleotide (16,17). In fact, proflavines' capacity, in this regard, was used in 1961 to discover the triplet nature of the genetic code.

In searching for effective anticancer and antitumor agents, advantage is taken of the fact that compounds which may interfere with cell replication at the molecular level may have medicinal utility. As a result, a large number of acridine derivatives have been investigated, only to find that, while a select few do have antineoplastic



properties, their associated toxicities to the host make them less desirable than other available compounds. On the other hand, aminoacridines have enjoyed success as antimalarial agents and are still in limited use today. Investigations were begun in the 1920's for a substitute for quinine, as an antimalarial, which led scientists first to methylene blue, a phenothiazine dye, then to pamaquine, both of which had low effectiveness against acute stages of malaria. Structural similarities in the above two compounds, with substituted acridines, eventually led investigators to quinacrine(VI) which became the drug of choice throughout World War II. Later, the more effective and less toxic chloroquine(VII) was discovered. Presently, considerable synthetic research is devoted to acridine-derived antimalarials to supplant chloroquine and to combat chloroquine-resistant P. falciparum -- discovered in 1961 (18). To date, the most promising compounds of this type have a diamine side chain in the 9-position(VIII). Structure-activity relationships for a variety of quinacrine analogues having side chain variants, terminal amine variants, and aromatic substituent variants, as well as quinine related acridine antimalarials, are tabulated by Henry (19).

In light of the broad biological significance of the binding of aminoacridines to DNA and other biopolymers, in vitro and in vivo studies aimed at understanding the exact nature of the interactions have been undertaken.

The following paragraphs will briefly review several of the more important articles germane to our investigation. Those dealing with the modes of binding will be outlined first, followed by studies of the effects of the ionic medium (including possibly competing ions), with a review of papers involving theoretical discussions, third.

An excellent comprehensive article on the interactions of aminoacridines with nucleic acids and the methods by which they may be studied was published in 1956 by A.R. Peacocke and J.N.H. Skerrett (20). Another, in 1968, by Blake and Peacocke (21) considered developments in the field after 1956.

Aminoacridines have long been known to bind to double-stranded DNA via two distinct modes -- termed type I and type II binding. Type I is the stronger and was first proposed by Lerman (22), to be an internal or intercalative process. Insertion of a molecular ion is accomplished by transient rotation of base pairs allowing the flat, lipophilic, aromatic portion of the molecule to become situated between them. The charged portion of the molecule was considered to remain external to the helix. H.J. Li and D.M. Crothers (23) corroborated this model when they presented indirect evidence that the base pairs remain perpendicular to the helix axis upon intercalation of the molecule. Another model for type I binding was advanced by N.J. Pritchard, A. Blake, and A.R. Peacocke (24) in which the small molecule is intercalated between adjacent

bases on the same polynucleotide chain. In both of these models, the cationic heteroatom is associated with an indeterminate number of anionic phosphate moieties along the DNA backbone. The viscosity of heat-denatured DNA is essentially unaffected by binding of acridines (25) whereas the viscosity of native double-stranded DNA is increased. An increase in viscosity is indicative of a lengthening of the polymeric chain such as would result from intercalation between base pairs or between adjacent bases. Since the single-stranded DNA undergoes no viscosity change, the Lerman model appears more plausible.

Some specific geometrical parameters of substituted acridine-DNA intercalative complexes were established by G.R. Kelly and T. Kurucsev (26) through the evaluation of linear dichroic spectra of stretched DNA films. An advantage of films over conventional methods involving aqueous solutions is that they do not require large ratios of drug to DNA phosphate. Taking advantage of the dichroism of two mutually perpendicular polarized transitions of the 9-aminoacridine nucleus, the workers were able to assess the angles of tilt and twist of the plane of the dye relative to the polymer helix. The long axis of acriflavine(II) was essentially perpendicular to the helix axis ( $85 \pm 5^\circ$ ), proflavine(III) was tilted slightly ( $77 \pm 2 \frac{1}{2}^\circ$ ), while 9-aminoacridine(IX) was tilted significantly ( $67 \pm 3^\circ$ ).

Type II, surface, or external binding has come to be generally accepted as an association of the small molecules with the exterior surface of the DNA polyanion. This interaction is, consequently, more strongly affected by alterations of the solvent system than is type I binding. Li and Crothers (23) determined, on the basis of temperature-jump kinetic data, that proflavine binds both internally and externally to calf thymus DNA and only about 7% of the total amount bound binds externally in the presence of 0.2M Na<sup>+</sup> (sodium phosphate - sodium nitrate). Decreasing the sodium ion concentration to 0.02M results in about 30% of the total being externally bound.

A careful study of the intercalative binding of a series of aminoacridines was conducted by Drummond and co-workers (27). Binding decreases in affinity in the order: acranil(X) > neomonacrin(XI) > atebrin(VI) > 9-aminoacridine(IX) > proflavine(III) 9-amino-1,2,3,4-tetrahydroacridine(XII). This sequence shows that side chain, and ring, substituents are not predominant in influencing binding affinity. The effect of greatly decreasing the planar area of 9-aminoacridine by hydrogenation is evident upon comparing its position with that of XII. The authors discuss various geometries of binding and conclude it is not necessary that there be exact and complete intercalation for type I (strong) binding. Rather, a modified Lerman model in which the charged heterocyclic ring nitrogen interacts with a DNA phosphate while the

rings only partially interact with the bases is more in accord with observed behavior.

It has been shown that the degree of intercalation may be dependent on the base composition of the DNA. Heterogeneity of intercalative sites was demonstrated by J.C. Thomes, G. Weill, and M. Daune (28) using fluorescence quenching techniques. They demonstrated that between 2% and 3% of the base pairs form sites where proflavine is strongly bound while the remainder have binding constants 3 to 4 times weaker. Proflavine fluorescence is relatively unaffected in the former case, but is totally quenched in the latter. The stronger sites correspond to adenine-thymine rich regions of the DNA indicating proflavine's specificity for the base pair. Similarly, Ramstein and Leng (29) showed that the location of bound proflavine, within the DNA, was dependent upon the base composition. The absorptiometric and fluorimetric titrations of DNA and partially methylated DNA (18%, mainly on N<sup>7</sup> of the guanine residues) indicated acridine was intercalated in both the native and methylated sites. However, greater apparent equilibrium constants for the methylated DNA than those for the native type were observed at two ionic strengths:  $14.25 \times 10^4 \text{ M}^{-1}$  and  $43.5 \times 10^4 \text{ M}^{-1}$  in 0.1M NaCl and  $40 \times 10^4 \text{ M}^{-1}$  and  $83.5 \times 10^4 \text{ M}^{-1}$  in 0.01M NaCl, respectively. These differences may be due to an increase in the distance between base pairs arising from the presence of the methyl groups which may, in turn, result

in the shift in the equilibrium concentrations. S. Georghiou (30) obtained much the same results as Ramstein and Leng based on the fluorescence decay of proflavine when it was bound to DNA at a phosphate to drug ratio of 420. Gabbay and co-workers (31) invoked the "Bookmark" model of intercalation to postulate ten possible distinct sites of binding of N-substituted-N-methylphenanthroline cations(XIII) to nucleic acids. They also investigated the topography of some nucleic acids in solution using PMR (32).

Binding models for the interactions of acridine orange(XIV) and proflavine(III) with DNA at ionic strengths of 0.002M, 0.020M, and 0.20M (tetramethylammonium cacodylate buffer, pH 6.5, 22°C) were proposed based on thermodynamic, spectroscopic, and hydrodynamic properties of the systems (33). The methods precluded consideration of surface binding. Binding isotherms were generated from equilibrium association constants which were, in turn, obtained from theoretical mass action relationships. Additional information, useful in developing binding models, was afforded by Goswami, Das, and Das Gupta (34) who measured the decrease in the static dielectric constant of a proflavine-DNA complex while decreasing the DNA phosphate/proflavine (P/D) ratio. The authors confirmed that: the bound dye neutralizes the charge on the DNA polyanion; at high P/D values, intercalation predominates and; that when P/D becomes small ( $\leq 10$ ), a cooperative



increase in the electrostatic binding of phosphate groups brings about a rapid decrease in the total surface charge of the polymer. In brief, at P/D values between 10 and 100 most of the cations are intercalated with a concomitant elongation of the polyanionic DNA helix, whereas below P/D of 10, surface binding is predominant. In the latter case, the spatial extension of the helix is decreased due to charge neutralization with no accompanying expansion due to intercalation. These results indicate that alterations in the dielectric constant of a medium containing such a system would manifest dissimilar effects on the two equilibrium association constants.

The specific effects of cations, other than the dye species, on the reaction between DNA and a dye is an integral part of this investigation. Therefore, a brief consideration of articles dealing with the binding of metal cations to DNA and related topics is in order. A tabulation of sites and thermodynamic quantities associated with proton and metal ion interactions with DNA, RNA, and their constituent bases, nucleosides, and nucleotides has been published by Izatt et al., (35).. H. Sigel and D.B. McCormick (36) provide an introduction to the fundamental theories concerning metal-ligand interactions in biological systems, including discussions of the Irving-Williams series, competition of metals and protons for sites, the formation of ternary complexes, and effects of solvent polarity and ionic strength. The Pearson theory (37) of



hard and soft acids and bases is applied to metals and polymers in relation to the biological roles of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{++}$ , and  $\text{Mg}^{++}$  and to the carcinogenicity of heavy metals (38).

The relative effectiveness of ions in altering the conformation of macromolecules via specific ion interactions is termed the Hofmeister, or lyotropic, effect (39). Generalizations based on observations of simple, ideal polymers may be confidently extrapolated to more complex polymers since it has been shown that the Hofmeister effect of a given ion is remarkably independent of the nature of the macromolecule. Moreover, while changes in the entropies of polymer segments are affected by both anions and cations, as are changes in the partial molal volume, the partial molal internal energy of a segment is dependent only on cations. Considering the above, M.J. Hey, J.M. Clough, and D.J. Taylor (39) reported that the ion binding strength with protein is (in decreasing order of strength)  $\text{NH}_4^+ < \text{K}^+ < \text{Na}^+$  and  $\text{Na}^+, \text{Li}^+ < \text{H}_2\text{O} < \text{Ca}^{++}$ . This series may be useful in separating solvent effects from ion binding effects where the above cations are present along with 3-aminoacridinium cation and DNA.

Stabilizing effects of various ions on four biopolymers, including DNA, were evaluated by Hippel and Schleich (40). The effects were discussed in terms of free energy of transfer for model compounds, activity coefficient variations, and correlations between ion effects on

macromolecules and on water structure. The relative effectiveness of the ions studied, in increasing the stability of the native configuration of DNA, paralleled the Hofmeister series:  $(\text{CH}_3)_4\text{N}^+ > \text{K}^+ > \text{Na}^+ > \text{Li}^+$  and  $\text{Cl}^-$ ,  $\text{Br}^- > \text{CH}_3\text{COO}^- > \text{ClO}_4^- > \text{CNS}^-$ . It was found for a series of tetraalkylammonium salts, the greater the length of the alkyl chains, the lower the stabilization of the native form of DNA.

Hen and Strauss (41) employed equilibrium dialysis, dilatometry, and viscometry to assess the counterion binding of a series of cations with poly(vinylsulfonate). The Hofmeister series was maintained (in order of decreasing binding affinity):  $\text{Ag}^+ > \text{K}^+ > \text{Na}^+ > \text{H}^+, \text{Li}^+ > (\text{CH}_3)_4\text{N}^+$  and  $\text{Ba}^{++} > \text{Mg}^{++}$ . All of the cations except  $\text{H}^+$  and  $\text{Li}^+$  are capable of cross-linking the polyanion through simultaneous site binding at two sulfonate groups.

The specific interactions of univalent and divalent cations with calf thymus DNA can be correlated with their respective unhydrated ionic radii (42,43). The order of most weakly bound to most strongly bound is:  $(\text{CH}_3)_4\text{N}^+ < \text{K}^+ < \text{Na}^+ < \text{Li}^+ < \text{Ca}^{++} < \text{Mg}^{++} < \text{Mn}^{++}$ . Comparing binding parameters with DNA with polyphosphate as polyanion, the authors showed that the sites on the biopolymer were the phosphates rather than bases or base pairs. These results were in agreement with those of other workers (44,45).

Conductometric titrations of DNA with divalent cations were first performed by J. Shack, R.J. Jenkins, and J.M. Thompsett (46) who found a sharp end point corresponding to 0.8 equivalents of  $Mg^{++}$  and  $Ca^{++}$  per mole of polymer phosphate. Similar titration behavior was seen for poly A and poly U by Felsenfeld and Huang (47) who developed an improved binding model which showed that the apparent stoichiometry of 0.8 was in error and that the true equivalence point corresponded to 1.0 eq of  $Mg^{++}$  or  $Ca^{++}$  per mole of DNA phosphate. The authors also disproved the previously held belief (48) that dications are more strongly bound to purines than pyrimidines in a polynucleotide.

It has been reported (49) that there is binding to base pairs of the DNA helix as well as to phosphates. The investigators reported that the effects of adding  $Mg^{++}$  to heat-denatured calf thymus DNA was to produce an absorption spectrum similar to that of denatured DNA alone, except for reduced absorbance. However, when  $Mg^{++}$  was added to native DNA, which was then denatured, a spectral shift to longer wavelength occurred. The authors concluded, on these grounds, that the metal was binding only to phosphates in the former case while in the latter, there was interaction with the aromatic nitrogens of the bases. The bonding between  $Mg^{++}$  and the nitrogens is presumably not able to occur unless the hydrogen bonds between the bases are broken in the presence of the metal.

Mathematical relationships incorporating changes in activities of ions in solution and deviations in activity coefficients were developed by Lyons and Kotin (45) to qualitatively distinguish between specific (site) binding and non-specific (diffuse electrostatic) binding of metals to polyanions. The rule of additivity of the activities of counterions of solutions of polyelectrolytes and simple salts was re-examined. It was found that, under certain conditions, there is significant exchange between the counterions associated with the polyanion and those of the bulk solution. A decrease in the activity coefficient of sodium ion, upon dilution of the system, was observed, indicating increased binding at lower concentrations. In the case of magnesium the results were not as simple but suggested that at high magnesium ion concentrations, specific site binding was predominant while at low concentrations, non-specific binding obtained. The differences between  $\text{Na}^+$  and  $\text{Mg}^{++}$  may be due, in part, to the ability of the latter to bridge between two adjacent anionic sites on the polymer whereas the monovalent cation cannot. Note that the distance between adjacent phosphates along the DNA backbone is approximately  $7 \overset{\circ}{\text{A}}$ . C.Sander and O.P. Ts'o (50) believe that the binding of  $\text{Mg}^{++}$  to DNA, RNA, poly A, poly A·U, poly I, poly I·C, and denatured DNA can be rationalized using a linear Scatchard relationship (vide infra). Their specific site model was applicable for up to about 70% saturation.

The degree of binding of acridine orange to native and denatured DNA was determined by equilibrium dialysis in 0.1M and 0.001M NaCl at 20°C (51). Three successive stages of binding were observed: the first corresponding to intercalation of monomers; the second, formation of bound dimers; and the third, external binding of aggregates. The appearance of the three modes of binding compared to two for most aminoacridines is attributed to acridine orange's proclivity to dimerize. The dialysis data was augmented with absorption and fluorescence spectra, electric dichroism, electric birefringence, and circular dichroic spectra.

Scruggs and Ross (42) found that the intrinsic viscosity of three types of DNA (salmon sperm, calf thymus, and T4 phage) decreases in the presence of univalent cations as the ionic strength increases, attaining separate limiting values for each. Prior to their work, the apparent insensitivity of the viscosity of DNA to variations in ionic strength was considered anomalous because polyelectrolyte viscosities are usually highly dependent on salt concentration. The workers asserted that previous invariance was due to the presence of trace amounts of basic proteins, polypeptides, and polyamines and that DNA was, in fact, similar to all other polyelectrolytes.

S. Bram (52) investigated the secondary structure of DNA in solutions of varying concentrations of  $\text{Li}^+$ ,  $\text{Na}^+$ ,  $\text{Rb}^+$ , and  $\text{Cs}^+$  using X-ray diffraction spectroscopy.

Differences between scattering patterns by sodium DNA in solution and those expected from theoretical calculations based on an unperturbed B form of DNA were due to structural changes in the B form arising from the polymer's interaction with the species in the medium. This is in concert with others' (53-55) conclusions that the number of base pairs per turn of the DNA helix is a function of the ionic environment. For cesium DNA the extrapolated lengths for 10 nucleotide pairs are  $43.5 \text{ \AA}$  at zero ionic strength and  $34.6 \text{ \AA}$  at finite CsCl concentration, whereas in NaCl, the values are  $33.5 \text{ \AA}$  and  $32.0 \text{ \AA}$ , respectively. (A novel DNA conformation model which differs markedly from the classical Watson-Crick B form is proposed by Stig Erlander (56) who argues that the differences between the values given above cannot be rationalized using the Watson-Crick model.) A theoretical treatment of cooperative binding was advanced by G. Schwarz (57) in which nearest neighbors along a linear polymer interact with one another. Two types of intrinsic internal binding are assumed: (1) that of an isolated ligand (nucleation); (2) that of a ligand to a site immediately adjacent to one already occupied. The model also includes considerations for dimer formation and competitive (non-cooperative) binding at adjacent sites by unrelated ligands. Matrix methods of calculating species concentrations under equilibrium conditions are presented.



An understanding of the effects of ionic strength on the binding of small molecules to DNA requires at least some knowledge of the charge distribution on the surface of the polyanion. Electrophoresis and membrane equilibrium experiments allow the determination of the apparent fractional charge per DNA phosphorous,  $i$ , which is an index of net charge (58). The value of  $i$  increases from 0.250 to 0.34 on going from a solution containing 0.005M NaCl to one having 0.05M NaCl, showing that the net charge of the polyanion increases with increasing ionic strength. Ross (58) considered the above information in light of Gorin's model of a long rigid cylinder (59) and applied it to DNA, which is intermediate between a rigid cylinder and a random-coil polyelectrolyte. Ross' data substantiates Gorin's assumption that a rigid cylinder (and, therefore, DNA) obeys Debye-Hückel approximations.

Manning (60) postulated that, as the ionic strength approaches zero, the effective charge of a polyelectrolyte is maintained at a critical constant by condensation of counterions along its surface as long as the formal charge does not exceed a critical value. His predictions were borne out by independent investigators (33) who found that at low ionic strength (0.002M) the electrophoretic mobility of DNA was unaffected by bound acridine orange.

Lerman's model for intercalation of small molecules into DNA satisfies many experimental observations but leaves some uncertainties unresolved arising from



consideration of the energetics involved (61). One of the most perplexing problems is the strong dependence of the intercalative mode of binding on the ionic strength (albeit not as dependent as surface binding). Also, the existence of a maximum degree of association as a function of ionic strength requires explanation. Attempts have been made to resolve these problems by invoking "stacking energies" rationalized in terms of short range, ionic strength independent interactions between DNA base pairs and the aromatic portions of the acridines. These rationalizations, however, are incompatible with the fact that aromatic hydrocarbons intercalatively bind only very weakly. M. Gilbert and P. Claverie (61) point out that the solution energies of the intercalating compounds parallel their binding affinities and, therefore, may be at least as important as the stacking energies. Moreover, the limiting of intercalation processes long before all available sites have been occupied mandates a limitation mechanism independent of the heterogeneity of sites. The critical effect of transferring positive charges from solvent "cavities," having dimensions on the order of the small molecules', into large cavities interior to the lipophilic DNA must be accounted for in a model of the dye-DNA complex -- as it is in their model.

T. Herskovits (62) studied the relationship between electrolyte concentration and solvent denaturation transitions in various media. Both increasing chain length and

increasing hydrocarbon content increase the effectiveness of the denaturant. It was concluded that these observations demonstrate the importance of hydrophobic forces in maintaining DNA's aqueous configuration. In most cases, the denaturation was reversible with added electrolyte.

It is Bradley's opinion (63) that by using a combination of statistical and quantum mechanical methods, it should be possible to relate all absorption, emission, equilibrium constant, and optical rotation observations in a unified way. With this aspiration in mind, he presented a statistical model of dye-polymer binding as a step toward that goal. He assumed that specific polymer sites were in a linear array and dye molecules were bound to them by simple electrostatic forces, with possible enhancement by neighboring sites. Furthermore, the sites were sufficiently close to allow bound dye-bound dye interactions with concomitant changes in their absorption, fluorescence, and phosphorescence spectra. The distribution of  $N$  consecutive sites occupied by dye molecules, in their varying states of aggregation, can be computed theoretically in terms of either the ground state free energy of interaction of a pair of neighboring dye molecules,  $\Delta F$ , or in terms of a stacking coefficient,  $K$ , related to  $\Delta F$ ,

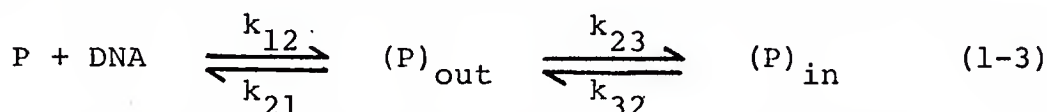
$$K = \exp(-\Delta F/kT) \quad . \quad (1-1)$$

The  $\Delta F$  and  $K$  terms may be equated to an experimentally obtainable ratio of  $P$  sites per  $D$  dye species,

$$P/D = (1-F^{1/2})^{-1}(1+(K-1)F) \quad (1-2)$$

Values of K range from 1.25 for DNA to 826 for polyphosphate ( $K = 6.2$  for denatured DNA). There is evidence that the value of K is directly related to the conformation of the polymer. The fact that K is equal to 1.25 and not to 1.00 for DNA -- as it would be for a completely random site occupancy -- indicates some non-randomness which could arise from dye-dye interaction along the polymer. A simplified quantum mechanical treatment is presented to allow computation of the magnitudes of the relationships between the dye molecules of filled units with empty sites.

Kinetics of intercalation of aminoacridines into DNA were first studied in depth by Li and Crothers (23) in 1969. Employing data from temperature-jump relaxation methods, they proposed a relatively simple two-step process for intercalative binding. It is worth noting that static experiments will not allow a separation of these two steps of the intercalative mode. The data fit the mechanism



where P is proflavine and  $(P)_{\text{out}}$  and  $(P)_{\text{in}}$  are surface bound and intercalatively bound dye, respectively. The overall insertion reaction occurs in the millisecond time range and is first order from the  $(P)_{\text{out}}$  state. The two-step hypothesis is substantiated by comparing the large

relative thermodynamic and spectral changes of the exterior binding with the small changes for the interior-bound species that occur upon glucosylation of the DNA (vide infra). Two limiting mechanisms are advanced and alternate mechanistic pathways discussed.

Passero, Gabbay, and others (64) used a simplistic model which included competitive effects of metal ions in solution to arrive at apparent association constants of two reporter molecules (XIIIa, XIIIb) to DNA. Electrostatic potentials and interactions between near neighbors were ignored, as were any forms of surface binding by the reporter molecules. The overall binding process was expressed as two separate equilibria



and



where R, M, and P are free reporter, metal, and DNA phosphate, respectively, and RP and MP the corresponding bound species. An apparent binding constant,  $K'_R$  may be defined relative to the metal ion concentration

$$K'_R = [RP] / [R] [P] \quad (1-6)$$

Finally, assuming the binding of R or M to the polyanion results in only one phosphate being affected per R or P, the apparent association constant may be expressed in terms of association constants for reporter binding,  $K_R$ , and

metal binding,  $K_M$

$$K'_R = K_R / (1 + K_M [M^+]) \quad . \quad (1-7)$$

The authors maintain that their assumptions are valid by virtue of the fact that they obtain reasonably constant values of  $K_R$  and  $K_M$  under a variety of conditions. However, if the reporter molecules are intercalatively bound, as the authors assert (and they are probably correct) then it is not valid to compare the reporter and metal binding because two entirely different types of sites are being occupied. The highly hydrophilic, symmetrically charged metal cations are almost certainly surface bound. Thus, the constancy of the results are probably fortuitous or, at best, important deviations are being masked by experimental error.

Relaxation kinetics of ethidium bromide(XV) binding to DNA revealed three types of bound species at equilibrium (65). Aside from the conventional surface-bound and intercalated species ascribed to type II and type I binding, a third species was found which involved an ethidium bromide transiently attached to sites of two separate DNA strands. The bimolecular rate constant for transferring an ethidium bromide from one DNA phosphate to another is 3 to 6 times larger than the rate of intercalation of free molecules. The more rapid bimolecular mechanism is dependent on the small molecule's ability to bind to two sites simultaneously. In the case of ethidium bromide, the tricyclic region of the

molecule provides one site while the phenyl moiety is believed to provide the second, less favorable, site.

Divalent-cation-specific electrodes were used to study the binding of  $Mg^{++}$  to DNA (50) in a solvent containing  $5 \times 10^{-3} M$  pH 7 phosphate buffer. Results were treated in terms of site-binding to phosphates in a multiple equilibrium process, employing a Scatchard treatment, which showed linearity over a range of 30% to 70% saturation of sites. Approximations to the Scatchard method yielded an apparent intrinsic binding constant of  $6 \times 10^3$  ( $\Delta G^0 = 5.2$  Kcal/mole). The use of cation-specific electrodes mandated DNA phosphate concentrations in excess of  $5 \times 10^{-3} M$ , causing aggregation of DNA and precipitation of MgDNA. Thus, the values of the constants may include significant error.

Three methods for evaluating equilibrium association constants for the binding of small molecules to DNA will be outlined. They are, the classical and most commonly employed Scatchard method, a modified Scatchard model, and a mass-action approach which will be used for this investigation. Scatchard's original treatment was designed for binding of small molecules to proteins. In Scatchard's words:

If the various groups on a protein molecule act independently, we can apply the Law of Mass Action as though each group were on a separate molecule and the strength of binding can be expressed as the constant for each group. Often a single constant will express the behavior of several groups. (66:660)

If the groups do act independently of one another, changes



in free energy for the reaction of the protein with small molecules is made up of the statistical entropy terms for  $v$  plus a term proportional to  $v$ . When the total number of sites (or groups of sites) is large, the calculations become tedious -- if the total number is not known the calculations cannot be performed. Such a situation does arise if one attempts to apply the equations without modification to nucleic acid binding. Since DNA polyanions commonly have molecular weights in excess of one million and may contain as many as  $10^6$  phosphates (67) Peacocke and Skerrett (20) redefined the parameters of the Scatchard equations to allow their application to nucleic acids. Their assignments and an abbreviated derivation of the Scatchard equations are as follows.

Let  $R$  represent the amount of small molecules,  $D$ , bound per mole of nucleic acid phosphorus (generally a fraction), and  $C$  represent the molar concentration of free  $D$ . Consider a number,  $n_j$ , of  $P$  classes of binding sites for  $D$  on the DNA polyanion. If each class has associated with it an intrinsic binding constant  $K_j$ , then we may write for any general case

$$R = \sum_{j=1}^{J=P} \frac{n_j K_j C}{1 + K_j C} \quad (1-8)$$

This may be simplified when all sites are identical

$$R/C = K_n - K_r \quad (1-9)$$



If there are two distinct types of binding sites, I and II, then

$$R = \frac{n_I K_I C}{1 + K_I C} + \frac{n_{II} K_{II} C}{1 + K_{II} C} \quad (1-10)$$

Figures 2a and 2b are representative plots of  $R/C$  vs  $C$  for one and two types of sites, respectively. Curvature may result in the case of only one type of site from variations in the electrostatic free energy term which is dependent on  $R$ . Such non-linearity can become critical when the intercalating species bear opposite charges but may be partially obviated by replacing  $K_I$  with the term

$$K'_I \exp (\Delta G_r^O / (RT)) \quad (1-11)$$

where  $K'_I$  is independent of  $R$ , and  $\Delta G_r^O$  is an electrostatic free energy term dependent only on the total effective potential. The case in which there are two types of binding sites is of primary interest in this work. The stronger, intercalative binding corresponds to type I of figure 2b whereas surface binding is represented by region II. With two types of sites, it is sometimes possible to discern distinct linear portions in the plots of  $R/C$  vs  $R$  as suggested in the idealized figure. Only if the magnitude of one of the constants is about 10 to 100 times greater than the other, can reliable values of the individual association constants be obtained by extrapolation of the data in each of the linear regions. Even then,

potentially erroneous assumptions regarding changes in the electrostatic free energy must be considered. Moreover, as previously stated, the proposed model does not allow for any form of interaction between the two types of binding sites. This is not generally serious in protein binding where the sites are often noninteracting but can be a problem in the case of the nucleic acids. Factors such as cooperative binding and near-neighbor exclusion will introduce sigmoidal characteristics into the plots of  $R/C$  vs  $R$  (21). Another shortcoming of the Scatchard method is that extrapolation of the legs of the hyperbolic plot to obtain slopes and intercepts often places an inordinately high degree of reliance on the data at the extrema of titrations. It is at the extrema that the data, regardless of how it was obtained, will be the least precise.

For aminoacridines, intercalative binding predominates up to a value of  $R \leq 0.20$  corresponding to 4 or more phosphates per bound cation. Surface binding, on the other hand, is predominant for values of  $R$  between approximately 0.5 and 1 for compounds which do not dimerize and up to 2 or more for those that are known to aggregate as free species at moderate concentrations.

Numerous investigators have suggested improvements of the basic Scatchard method as modified by Peacocke and Skerrett. Included are corrections for electrostatic effects (27,31,61,63), competition of metallic ions in

solution (45,46,67), near-neighbor effects (23,50,57, 63,65), small molecule aggregation (51,57), and configurational effects due to solvent and ionic factors (23,25,28, 39,44,52,62,68). One of these improved models, proposed by Armstrong, Kurucsev, and Strauss (33) incorporates some novel features including: (1) the total concentration of intercalation sites available is fixed, a priori, to include every slot between successive DNA base pairs; (2) an intercalated dye inhibits intercalation at its two immediately adjacent slots; and (3) inclusion of a term to allow for an intercalated monomer forming a dimer with a free monomer resulting in a spectroscopically distinct species. The authors found (3) became important when the degree of binding,  $\beta$ , exceeded about 0.2 mole of dye per mole of DNA phosphate (dyes were acridine orange and proflavine). Their expressions for the association constants, based upon theoretical mass action relationships are, for intercalation

$$K_1 = \frac{2(\beta_1 - \beta_2)(1 - 2\beta_1)}{C_M(1 - 4\beta_1)^2} \quad (1-12)$$

and for surface binding

$$K_2 = \frac{\beta_2}{C_M(\beta_1 - \beta_2)} \quad (1-13)$$

where  $\beta_1$  = the number of intercalated molecules per DNA phosphate.

$\beta_2$  = the number of externally bound dimers per DNA phosphate.

$C_M$  = the molar concentration of free dye monomer.

$K_1$  = the apparent binding constant for the monomer species intercalating.

$K_2$  = the apparent binding constant for surface binding.

These relationships imply that the electrostatic potential of the DNA is unaffected by binding. Values of  $\beta_1$  and  $\beta_2$  may be spectroscopically evaluated knowing the molar absorptivities of the bound monomer,  $\epsilon_M$ , and the bound dimer,  $\epsilon_D$ ,

$$\epsilon_T = (\epsilon_M \beta_M + \epsilon_D \beta_D) / \beta \quad (1-14)$$

where  $\beta_M = \beta_1 - \beta_2$ ,  $\beta_D = 2\beta_2$ , and  $\epsilon_T$  is the total "molar absorptivity" of all bound species. The model was tested by determining the binding of acridine orange and proflavine to DNA (pH 6.5, 22°C) at ionic strengths of 0.002M, 0.020M, and 0.200M. The above method is an improvement over the Scatchard procedure but still demands a priori selection of an exact mode of reaction. That is, though the overall stoichiometries are experimentally evaluated, the thermodynamic manner in which the free species react is not defined. For example, in the intercalative case, where 4 phosphates are involved for each 3-aminoacridinium binding site, are the 4 phosphates to be considered as 4 separate entities, as 2 separately interacting moieties,

each containing 2 phosphates, or as one entity containing all 4 phosphates? In each case, the overall stoichiometry is 4 to 1, but the equilibrium expression of the first includes a fourth power term in DNA phosphate concentration, the second would have a squared phosphate concentration term, while the third would be linear in phosphate.

To resolve this problem, Capomacchia (69) has proposed a mass-action approach based on best-fit analyses of spectroscopically derived concentrations of free and bound species. Assuming that the binding process obeys the Law of Mass Action and total concentrations of all species are sufficiently low that aggregation of like species is insignificant, we may write



where BH is the free cationic form of the small molecule, q is the number of DNA binding sites that complex one small molecule and Su denotes the unbound DNA binding sites. Each site, Su, acts as one distinct entity in the equilibrium expression and may contain any number (1,2,3,. . . m) of phosphates. Note that throughout this text binding sites will be discussed in terms of DNA phosphates, simply for convenience, because the total concentration of DNA phosphate is easily obtained experimentally. This terminology does not imply, for instance, that phosphates are the binding sites for intercalatively bound drug. The association constant,

on a molar basis, for the process is

$$K = [\text{BHP}] / [\text{BH}] [\text{Su}]^q \quad (1-16)$$

where  $[\text{BHP}]$  is the molar concentration of bound species,

$[\text{BH}]$  is the concentration of free small molecule, and

$[\text{Su}]$  is the concentration of the uncomplexed DNA sites.

Unbound site concentration may be determined from the

total site concentration,  $S_t$ , and bound site concentration,  $S_c$

$$S_u = S_t - S_c \quad (1-17)$$

Now, since

$$S_c/q = [\text{BHP}] \quad (1-18)$$

$$\text{and } S_t = [P_t/m] \quad (1-19)$$

where  $P_t$  is total DNA phosphate concentration, we may write

$$S_u = [P_t/m] - q[\text{BHP}] \quad (1-20)$$

Substituting equation (1-20) into (1-16) yields

$$K = \frac{[\text{BHP}]}{[\text{BH}] ([P_t/m] - q[\text{BHP}])^q} \quad (1-21)$$

Taking the log of both sides of (1-21) and rearranging, we obtain

$$\log \left( \frac{[\text{BHP}]}{[\text{BH}]} \right) = \log K + q \log ([P_t/m] - q[\text{BHP}]) \quad (1-22)$$



Molar concentrations of free and bound small molecule species may be determined from any of a variety of experimental techniques. The overall stoichiometry of the process may be obtained from a Scatchard treatment or a Job's plot. Knowing the overall stoichiometry, initial values of  $m$  and  $q$  may be selected which satisfy the simple relationship  $mq = n$ , where  $n$  is the total number of DNA phosphates per bound small molecule. A rapid determination of the most probable combinations of values may be made by plotting  $\log ([P_t/m] - q[BHP])$  vs.  $\log ([BHP]/[BH])$ . That pair which yields the most consistent straight line having a slope of  $q$  may then be used to compute values of  $K$  for a series of ratios of  $[BHP]$  to  $[BH]$ . Again, the pair which provides the most consistent calculated equilibrium association constants for all data points, corresponding to either the surface or intercalative mode, are taken as the exponents in the binding expression. For interactions between 3-aminoacridinium cation and calf thymus DNA, best-fit values of  $m$  and  $q$  are 3 and 1, respectively for surface binding and 2 and 2 for the intercalative mode (69). To summarize, the method of Capomacchia allows for the determination of equilibrium association constants based on mechanistic considerations. Furthermore, by computing values of  $K$  from data in the midregion of a titration, undue reliance on the least trustworthy data at the extrema of a titration is avoided.

Many workers have been satisfied with reporting association constants calculated from molar quantities rather than

from the more correct activities of the various species. Doing so implies two things: activities of the various species in the expression are equal to molarities, and the molarities are equal to molalities. It is unlikely that approximating molalities with molarities introduces significant error throughout the usual concentration ranges employed. But activities, on the other hand, may differ markedly from molarities. For this reason, evaluation of association constants in terms of activities is recommended. Molality is related to activity by

$$a_i = \alpha_i m_i \quad (1-23)$$

where  $a_i$  is the activity,  $\alpha_i$  is the activity coefficient, and  $m_i$  is the molality of species  $i$ . Note that the activity equals the molality only when  $\alpha = 1$ . For a process in solution



the equilibrium constant is rigourously defined as

$$K = \frac{a_{AB_n^{-(n+1)}}}{(A^+) (B^-)^n} = \frac{\alpha_{AB_n^{-(n+1)}} M_{AB_n^{-(n+1)}}}{(\alpha_{A^+} M_{A^+}) (\alpha_{B^-} M_{B^-})^n} \quad (1-25)$$

It is extremely difficult to determine individual activities since there is no way of separating effects due to positive ions from those due to the accompanying negative ions. We may, however, define a mean activity,  $a^+$ , which is the geometric mean of the individual activities

$$A^{\pm} = (A^{+} \cdot A^{-})^{1/2} \quad (1-26)$$

Similarly,

$$\alpha^{\pm} = (\alpha^{+} \cdot \alpha^{-})^{1/2} \quad (1-27)$$

For ideal dilute solutions, the mean ionic activity coefficient will be equal to 1. If deviations from ideality are caused entirely by electrical interactions of point charges, it may be shown that, for aqueous solutions at 25°C (70)

$$\log \alpha^{\pm} = -.509 / [Z_{+} Z_{-}] I^{1/2} \quad (1-28)$$

where  $Z_{+}$  and  $Z_{-}$  are the charges of interacting species and  $I$  is the total ionic strength of the system

$$I = 1/2 \sum_i m_i Z_i^2 \quad (1-29)$$

Equation (1-28) is an expression of the Debye-Hückel limiting law for activity coefficients and is applicable only to dilute solutions containing point charges. As the ionic strength becomes large (e.g., >0.01m) and/or the ions become less ideal (become larger, more polarizable, etc.) the relationship becomes increasingly approximate. Under these conditions, an extended Debye-Hückel equation may be invoked which introduces a correction for the finite sizes of ions:

$$\log \alpha^{\pm} = \frac{-0.509 / [Z_{+} Z_{-}] I^{1/2}}{1 + B d I^{1/2}} \quad (1-30)$$

B is a constant for a given solvent and includes such terms as solvent viscosity, dielectric, and temperature while d is an average effective diameter of the ions. A factor not considered in equation (1-30) is the effect of ions on solvent molecules and solvent structure. This may become especially important for solutions containing large polyanions such as DNA.

Lyons and Kotin (44,45) report a method of assessing values of  $a^+$  and  $a^-$  independently of one another. They also determined  $\alpha^+$  for  $\text{Na}^+$  and  $\text{Mg}^{++}$  when binding to DNA and three synthetic polyanions. Their somewhat tedious procedure involving equilibrium dialysis allowed them to distinguish site-specific and non-specific forms of surface binding. The activity coefficient of  $\text{Na}^+$  was shown to decrease with increasing dilution of metal ion in the presence of DNA suggesting an increased degree of binding.

#### Thermodynamics of Binding Reactions

A second topic of this work is concerned with the thermodynamic parameters associated with the binding of small molecules to DNA. Hence, a brief review of several pertinent articles is presented here.

M. Gilbert and P. Claverie (61) measured total energies for an intercalation process and attributed them to three types of electrostatic interactions. They were (1) the attractions between the bound cations and the DNA phosphates, (2) the repulsive forces between cations, and (3) solvent

response to the electric field generated by the charged species. At high ionic strength the phosphates are relatively neutralized causing (1) to become insignificant and, thereby, causing a decrease in both the strength and extent of binding. Some limiting of the binding process occurs even at very low ionicities because cation-cation repulsions are still important with respect to intercalated species. The third type of interaction serves to reduce all forms of electrostatic energies and will be most effective for those species most intimately in contact with the solvent. The authors have shown that phosphates and complexed acridines are more solvated than free acridines so that, as the ionic strength is raised, their stabilizing interactions are more reduced than are acridine-acridine interactions. In that the forces arising from the latter are destabilizing, the result of the countervailing trends is a net destabilization of the complex with increasing ionic strength. The model used here can also be applied to other cationic small molecules, including protons. Similar results were reported for the binding of proflavine to DNA via fluorimetry (71). It was found that the electrostatic contribution to the total free energy decreased with increasing ionic strength due to screening of the potential by an ionic atmosphere.

Thermodynamic parameters accompanying the intercalation of calf thymus DNA were evaluated by Chambron, Daune, and Sadron (72) using equilibrium dialysis. Temperatures ranged from

0°C to 70°C and ionic strengths of 0.01M, 0.1M, and 1.0M were maintained using acetate buffer at pH 5.9 in the presence of EDTA. The number of sites available for binding decreased upon increasing either the temperature or the ionic strength. The enthalpy of reaction decreased with decreasing temperature;  $\Delta H^0 = -19$  Kcal/mole at 70°C and  $-4.5$  Kcal/mole at 0°C ( $I = 0.01$ ). This was ascribed to a thermally reversible change of state of DNA which occurs at approximately 40°C. Total standard free energy was considered to be a sum of three terms. The first of these,  $\Delta G_S^0$ , characterizes the dipole-induced dipole interaction between base pairs and cations while the second,  $\Delta G_e^0$ , is the electrostatic free energy. The third,  $\Delta G_H^0$ , is the solvation free energy corresponding to desolvation of reactants and solvation of the bound complex. At high ionic strengths,  $\Delta G_S^0$  and  $\Delta G_e^0$  are very small, hence, the total free energy of 6.4 Kcal/mole is due to  $\Delta G_S^0$ . The authors' value of  $\Delta S^0 = 11$  Kcal/mole degree is in agreement with theoretical predictions.

Thermodynamic energies of reaction between proflavine and two forms of DNA determined from temperature-jump relaxation methods are summarized below (23).

| DNA            | $E_{12}^+$ | $E_{21}^+$ | $E_{23}^+$ | $E_{32}^+$ | $\Delta H_{12}^0$ | $\Delta H_{23}^0$ | $T\Delta S_{12}^0$ | $T\Delta S_{23}^0$ |
|----------------|------------|------------|------------|------------|-------------------|-------------------|--------------------|--------------------|
| calf<br>thymus | 4          | 14         | 16         | 14         | -9.8              | 2.0               | -5.4               | 3.2                |
| T2             | --         | --         | 13         | 17         | -3.4              | -3.9              | 1.6                | -2.8               |

$E_{ij}^+$  is the activation energy for transformation from state  $i$



to  $j$  (see equation (1-13) and  $\Delta H_{ij}^{\circ}$  and  $\Delta S_{ij}^{\circ}$  are the thermodynamic enthalpy and entropy changes for the same reaction. Measurements were done over the temperature range  $10^{\circ}\text{C}$  to  $25^{\circ}\text{C}$ ,  $0.20\text{M Na}^{+}$  and pH 6.9. Of particular note here is that the enthalpy change for outside binding is much more strongly affected by glucosylation of the DNA than it is for the intercalated form (T2 DNA is glucosylated). For example,  $\Delta H_{12}^{\circ}$  changes from  $-9.8$  to  $-3.4$  Kcal/mole dye on going from calf thymus to T2 DNA but for the overall intercalation process ( $\Delta H_{12}^{\circ} + \Delta H_{23}^{\circ} = \Delta H_{13}^{\circ}$ ) the change is only from  $-7.8$  to  $-7.3$  Kcal/mole dye. It is unlikely that small differences in base composition between the two forms of DNA can account for the large differences in the thermodynamic values of the binding reaction.

Entropy and enthalpy changes during reaction of ethidium bromide with calf thymus DNA were computed from temperature-jump relaxation data by Bresloff and Crothers (65) over the temperature range  $15^{\circ}\text{C}$  to  $32^{\circ}\text{C}$  in  $1.0\text{M Na}^{+}$ . Values for the formation of a surface bound species were:  $\Delta H^{\circ} = 1.1$  Kcal/mole and  $\Delta S^{\circ}(19^{\circ}\text{C}) = 5.6$  Kcal/mole, while for one of a pair of intercalatively bound species:  $\Delta H^{\circ} = -7.8$  Kcal/mole and  $T\Delta S^{\circ}(19^{\circ}\text{C}) = -2.3$  Kcal/mole. Values for a second intercalated form could not be obtained due to the rapidity of its binding process.

Bradley (63) determined the ground state free energy of intercalation of a pair of neighboring acridine orange molecules bound to DNA to be  $-0.065$  Kcal/mole dye. By



comparing this to the spectral shift accompanying aggregation (504 nm to 464 nm) corresponding to 4.8 Kcal/mole dye separation in energy between the ground and excited states, he concluded that the spectral shift results from an increase in energy of the excited state, rather than a decrease in the ground state.

Enthalpy and entropy changes,  $\Delta H^{\circ}$  and  $\Delta S^{\circ}$  respectively, of reactions may be determined from the relationship between the equilibrium association constants for the processes at various temperatures and the temperature as set forth by the van't Hoff relationship.

$$\log K = \frac{-\Delta H^{\circ}}{2.303R} \frac{1}{T} + \text{Constant} \quad (1-31)$$

This important equation will be derived below (73).

For ideal systems, any change in the total free energy,  $G$ , can be said to be due to changes in either temperature or pressure, since the state function,  $G$ , can be defined in terms of pressure, volume, and temperature.

$$dG = \left( \frac{\partial G}{\partial P} \right)_T dP + \left( \frac{\partial G}{\partial T} \right)_P dT \quad (1-32)$$

We define

$$G = H - TS \quad (1-33)$$

and

$$H = E + PV \quad (1-34)$$

where  $H$  is the enthalpy,  $S$  is the entropy, and  $E$  is the

internal energy for the system under consideration. Recall that, the enthalpy changes are commonly termed "heats of reaction" for processes, entropy may be used as a measure of the degree of order or disorder, and that the internal energy is unavailable for work of any form. The change in the free energy,  $dG$ , may now be expressed as

$$dG = dE + PdV + VdP - TdS - SdT \quad . \quad (1-35)$$

For a reversible process in which no work other than expansion occurs,

$$PdV = dw \quad (1-36)$$

and

$$dE - dq + dw = 0 \quad . \quad (1-37)$$

The terms  $q$  and  $w$  refer to the heat absorbed, and work done, by the system respectively. Equation (1-37) is a direct consequence of the First Law of Thermodynamics and states, in effect, that the total change in the internal energy of a system is the sum (or difference) of the amount of heat absorbed,  $dq$ , by the system and the amount of work done,  $dw$ , by the system. Notice that heat and work are not functions of state (which are conventionally assigned upper case symbols). Equation (1-35) now becomes

$$dG = VdP - SdT \quad (1-38)$$

which upon equating with equation (1-32) yields

$$\left(\frac{\partial G}{\partial T}\right)_P = -S \quad (1-39)$$

and

$$\left(\frac{\partial G}{\partial P}\right)_T = V \quad (1-40)$$

Equation (1-39) suggests an experimentally tractable relationship between free energy (via  $K$ ) and temperature, when pressure is held constant. Moreover, we may extend the relationship to include the changes in free energy as a function of temperature for a process involving reactants going to products. We may now equate (1-38) with equation (1-32), rearrange, and solve for the partial differentials to obtain

$$(\partial \Delta G / \partial T)_P = -\Delta S \quad (1-41)$$

where  $\Delta G = (G_{\text{products}} - G_{\text{reactants}})$  and  $S = (S_{\text{products}} - S_{\text{reactants}})$ . The entropy term of equation (1-41) may be eliminated by recalling that  $\Delta S = (-\Delta H + \Delta G)/T$  and substituting

$$(\partial G / \partial T)_P = (-\Delta H + \Delta G)/T \quad (1-42)$$

Assume, henceforth, that the pressure will be held constant. Rearranging (1-42) gives

$$d\Delta G/dT - \Delta G/T = -\Delta H/T \quad . \quad (1-43)$$

Equation (1-43) may be expressed as

$$T \frac{d}{dT} (\Delta G/T) = -\Delta H/T \quad (1-44)$$

since, upon differentiation, it would become

$$T \frac{T(d\Delta G/dT) - \Delta G}{T^2} = \frac{d\Delta G/dT}{T} - \frac{\Delta G}{T} \quad . \quad (1-45)$$

Equation (1-45) may be written in terms of standard states, and, in that

$$\Delta G^{\circ} = -RT \ln K \quad (1-46)$$

it can be stated as

$$d \ln K/dT = \Delta H^{\circ}/RT^2 \quad (1-47)$$

which is equivalent to

$$\frac{d \log K}{d(1/T)} = \frac{-\Delta H^{\circ}}{2.303R} + \text{Constant} \quad (1-48)$$

Assuming that  $\Delta H^{\circ}$  is temperature independent, equation (1-48) can be integrated to yield equation (1-31). The above relationship may be applied to reactions in solution whose equilibrium association constants are based on the activities of the species involved [see equations (1-23) through (1-30)]. Plots of  $\log K$  vs.  $1/T$  should yield straight lines having slopes equal to  $-\Delta H^{\circ}/2.303R$ . If the relationship between  $\log K$  and  $1/T$  is not linear then the enthalpy of binding is

not constant with respect to temperature. For systems containing DNA, enthalpies of binding determined from van't Hoff plots are often not constant with temperature, but decrease with increased temperature, presumably due to changes in DNA's conformation (72). As a result, it should not be surprising that binding curves obtained spectrophotometrically and by dialysis equilibrium can agree at one temperature and disagree at another (21).

#### Rationale for the Selection of the Model System and Experimental Design

As this study is concerned with the effects of competing metal ions and of alterations of the ionic strength on the binding of small molecules to biopolymers, the choice of DNA as the model polymer is evident. The selection of 3-aminoacridine as the small molecule is based on several factors, two of which are especially important. The first criterion that the small molecule must meet is that it bind to DNA. To do so, it is necessary that the species exist as a cation since neutral compounds do not bind to DNA -- or if they do, the binding is too weak for our purposes. (It should be evident that electrostatic repulsive forces prevent anions from interacting with DNA.) Furthermore, the compound must be positively charged in solutions whose pH is between 4 and 11 so that denaturation of the polymer is minimal (74). Hence, if the compound is acidic its  $pK_a$  should be at least 5. The  $pK_a$  of 3-aminoacridine is 8.04 (7:Ch 4) meaning it is essentially totally protonated in a

solution of pH 6. Moreover, 3-aminoacridine allows convenient evaluation of both of the principal modes of binding of small molecules to DNA, under a given set of conditions, in a single titration. That is, the range of the ratio of total drug to total DNA concentration over which the two modes of binding can be experimentally evaluated is about 0.1 to 10. Obviously, it is mandatory that each type of binding occur in separate regions of the titration.

The second major criterion that the model compound must satisfy is that it allow an expedient means of measuring the extent of binding to the polyanion. Of the various methods of determining equilibrium constants one of the best is absorptiometric spectroscopy. The 3-aminoacridine cation is particularly amenable to this method for the following reasons. (1) It absorbs strongly in the visible region of the spectrum as the free cation having molar absorptivities of  $1.413 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$  at 365 nm and  $1.259 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$  at 454 nm (75). Such magnitudes allow good sensitivity at low concentrations of the compound. The molar absorptivities of the bound drug in this region are  $8.494 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$  at 368 nm and  $9.071 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$  at 463 nm. The large difference between the absorbances of the band maxima of the free and bound drug provides good sensitivity in following a titration. The longest wavelength absorption maximum for DNA is at approximately 260 nm meaning that there is little spectroscopic interference from the polymer. (2) Both the free and bound species have two distinct absorption

envelopes in the visible region corresponding to  $^1L_b$  and  $^1L_a$  bands [Platt's nomenclature (76);  $\alpha$  and para bands, respectively, in the Clar nomenclature (77)]. These arise from transitions from the  $^1A$  ground state to the  $^1L_b$  ( $\approx 365$  nm), and to the  $^1L_a$  ( $\approx 460$  nm) excited states. The transition moment of the former is parallel to the long axis of the acridine ring system while the moment of the latter is orthogonal to the  $^1L_b$  and parallel to the short axis of the molecule. By observing shifts in the two maxima, along with any changes in vibronic character, during the course of a titration, one may be able to deduce binding geometries (78). The 3-aminoacridine molecule also fluoresces in its free cationic form and, less intensely, as the bound species, thereby providing another spectroscopic method with which to study its interaction with DNA.

The more thoroughly studied aminoacridines, proflavine and acridine orange, meet the criteria mentioned above in regard to 3-aminoacridine. Both of them, however, begin to aggregate at concentrations as low as  $10^{-5}M$ , thereby introducing undesirable complications. Also, the mono-substituted 3-aminoacridine, being a simpler molecule than either proflavine or acridine orange, is preferable as a model compound. It is hoped that information gained using the simple compound may be applied to more complex systems.

Many references to the effects of ionic strength on the binding of small molecules to biopolymers can be found in



the literature. Unfortunately, the majority are ancillary to other studies and are not sufficiently complete in themselves to provide much specific information. Too, even for a given molecule binding to a given polymer, the wide variety of experimental variables under which the separate investigations were done precludes any valid comparisons of results. The present work was undertaken as a result of this lack of a comprehensive investigation. Our pharmaceutical interest led us specifically to DNA as the polyanion, though other, synthetic polymers may have provided a less complex system.

Some of the immediate questions which come to mind and which may be answered by an investigation of the effects of changing the ionic constitution of a drug-DNA solution are: are the effects predictable on the basis of electrostatic theory? That is, may the effects be explained in terms of the simple Debye-Hückel limiting law at low ionic strengths and by extended Debye-Hückel equations at higher ionicities? If the above questions cannot be answered affirmatively, might deviations at low ionic strengths be due to the polymeric nature of the anion? Are deviations at high ionic strengths due to specific ion interactions and, if so, can they be rationalized on the grounds of simple competition for binding sites or must a less simplified, mixed complex model be introduced? Other questions are: will altering the ionic medium affect surface and intercalative binding in the same manner? If not, how can the differences be reconciled?

How will changing the ionic radii, ionic charge, and charge density of the metallic ions affect the equilibria? The last question is particularly germane in in vivo biological binding studies because of the wide variations in concentrations of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{++}$ , and  $\text{Ca}^{++}$  in a living organism (79).

To try to answer these questions, equilibrium association constants for the surface and intercalative binding of 3-aminoacridinium cation to DNA in a variety of ionic media were determined. Background electrolytes included concentrations between 0.15M and  $6.3 \times 10^{-4}\text{M}$  of the phosphate salts of  $\text{Li}^+$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Rb}^+$ ,  $\text{Cs}^+$ , and  $(\text{CH}_3)_4\text{N}^+$ . Also considered were the acetates of the alkaline earths:  $\text{Mg}^{++}$ ,  $\text{Ca}^{++}$ ,  $\text{Sr}^{++}$ , and  $\text{Ba}^{++}$  at concentrations ranging from 0.025N to  $1 \times 10^{-5}\text{N}$ . The effect of  $\text{H}_2\text{PO}_4^-$  as opposed to  $\text{CH}_3\text{COO}^-$  as the counteranion of the supporting electrolyte was evaluated. The pH for all of the titrations was 5.90 and the temperature was  $25.0^\circ\text{C}$ . A minimum of two titrations were done for each set of conditions.

Thermodynamic parameters were evaluated for systems containing 3-aminoacridine and DNA with a background electrolyte of 0.010M  $\text{KH}_2\text{PO}_4$  at pH 5.90. Similar investigations were conducted using 7-aminoacridinium cation(XVI) in place of 3-aminoacridinium. By studying the binding of the linear benzalog of 3-aminoacridine and comparing the thermodynamic parameters of the two, the relative importance of enthalpy and entropy contributions to overall free energies may be assessed.

## CHAPTER II

### EXPERIMENTAL

#### Materials and Procedures

All chemicals were, unless otherwise stated, either reagent or analytical grade. Water was multiply distilled in a tin-lined still (Barnstead, Sybron Corp.). It was redistilled until its specific conductance was less than  $1.0 \text{ megaohm cm}^{-1}$  at  $25.0^{\circ}\text{C}$ . Stock solutions of supporting electrolytes were prepared within three weeks of use and were refrigerated during storage to minimize biological growth. Approximately 100 to 200 ml of a 0.15M solution of each electrolyte were prepared as outlined below and titrated with 0.72M  $\text{H}_3\text{PO}_4$ , 1M  $\text{CH}_3\text{CO}_2\text{H}$ , or 1M metal hydroxide solution to pH 5.9, in all cases. Concentrations of the hydroxide solutions of the various cations were determined by titration with standardized 1.000N sulfuric acid.

A stock solution of tetramethylammonium phosphate was made by titrating 13.16 ml of 1.14M tetramethylammonium hydroxide (Mallinckrodt Organic Reagent, 10% in water, lot: 15) in 70 ml of water with 0.72M phosphoric acid (Fisher, lot: 755459) and adjusting the final volume to 100 ml. Lithium phosphate stock solution was prepared by

titration of 0.476M lithium hydroxide with 0.72M phosphoric acid. The hydroxide solution was made by reacting stoichiometric amounts of anhydrous lithium sulfate (PCR, Inc., Gainesville, Florida, lot: 10465) and barium hydroxide heptahydrate (Mallinckrodt, lot: RTZ). Sodium and potassium phosphate stock solutions were prepared using sodium phosphate monohydrate (Mallinckrodt, lot: ABK) and monobasic potassium phosphate (Fisher Certified, Reagent, lot: 20175), respectively. The acid level of each solution was adjusted with 0.72M phosphoric acid to pH 5.9. The stock solution of rubidium phosphate was prepared by titrating an aliquot of 0.43M rubidium hydroxide (Pfaltz and Bauer, no lot number) with 0.72M phosphoric acid. Similarly, cesium phosphate was prepared from 0.201M cesium hydroxide (Pfaltz and Bauer, no lot number). Stock solutions of potassium acetate and tetramethylammonium acetate were prepared by titrating standardized aliquots of the respective hydroxides with 1M acetic acid (Baker Chemical, lot: 41397). Magnesium acetate solutions were made from magnesium acetate tetrahydrate (Mallinckrodt, control: NSB). Solid calcium acetate was made by dissolving calcium carbonate (Mallinckrodt, no lot number) in excess acetic acid. The calcium acetate was recrystallized twice from aqueous ethanol, washed repeatedly with 95% ethanol, and dried for twenty-four hours at 110°C to yield the monohydrate of the salt. The sample was found to contain 97.3%  $\text{Ca}(\text{C}_2\text{H}_3\text{O}_2) \cdot \text{H}_2\text{O}$ , by dissolving a known amount of the acetate

in water, precipitating the calcium as calcium carbonate and titrating the carbonate with hydrochloric acid.

Strontium acetate was prepared from its carbonate (Matheson, Coleman & Bell, lot: 19) and assayed in a manner similar to that for calcium acetate. It was determined to contain 95.0%  $\text{Sr}(\text{C}_2\text{H}_3\text{O}_2)$ . A recrystallized sample of barium acetate was dried for twenty-four hours at  $170^\circ\text{C}$  and assayed by forming barium carbonate. The solid carbonate was reacted with excess 1.00N sulfuric acid, carbonic acid removed by gentle heating, and the excess sulfuric acid determined by titration with standard sodium hydroxide solution. The assay of the barium acetate was 99.6%  $\text{Ba}(\text{C}_2\text{H}_3\text{O}_2)$ .

Solutions of the supporting electrolytes to be used as solvents were made from the stock solutions. Aliquots of the stock solutions were diluted to within 90% of their calculated final volumes and their acidities adjusted to  $\text{pH } 5.90 \pm 0.05$ , if necessary, using the appropriate metal hydroxide, phosphoric acid, or acetic acid. They were then diluted to their exact desired final volumes and their ionic strengths computed, taking into account any additional ions added to adjust the acid levels.

A commercially available sodium salt of DNA was dialyzed against a solution of the desired metal phosphate or acetate to obtain the DNA salts having the same cation as that of the supporting electrolyte. Solutions containing approximately 0.01 mole of DNA phosphate per liter were made

by placing 3.6 mg of DNA, sodium salt (Calbiochem calf thymus DNA, A grade, lot: 900007: 8.04% P; 12.21% N; moisture, 14%;  $E_{260nm}^{1\%} = 192$ ) per milliliter of solvent into twenty milliliter volumetric flasks. Between 5 and 20 ml of solvent were then added. The solvent was a 0.01M solution (pH 5.90) of the metal phosphate or acetate. The flask and its contents were placed on a vertical rotating mixer (Scientific Products model 150-V) operating at 6 rpm for about one hour, after which the sample was placed in an ultrasonic mixer for two to three minutes. Rotation and sonication were repeated for a total of at least five hours or well beyond the time at which the moderately viscous sample appeared homogeneous. The sodium DNA solution was then transferred to dialysis tubing (Union Carbide Films Packaging Division, 7.3 mm dia.). The tubing and its contents were immersed in a volume of 0.01M solution of the desired salt which was at least ten times greater than the volume of DNA solution being dialyzed. The external solution was agitated for two hours and then replaced. A total of six volumes of external solution were used for each dialysis procedure. The dialyzed DNA was again mixed for one hour on the rotating mixer and submitted to ultrasonication for two to three minutes. Then, the sample was divided among Pyrex<sup>R</sup> culture tubes (6x50 mm) each receiving approximately one half milliliter of sample. These were immediately stored at -5°C.



Determinations of the concentrations of DNA after dissolution and after dialysis yielded values which were identical, within experimental error and also within  $\pm 5\%$  of the calculated concentration based on the manufacturer's data. The agreement of these data were taken as evidence that any degradation of the biopolymer during the procedures was minimal.

The efficiency of the dialysis method, used here, to replace the sodium cations of the original DNA salt with the cations of the external solution was evaluated by atomic absorption spectroscopy. A Perkin-Elmer model 290B atomic absorption spectrophotometer outfitted with a combination sodium/potassium lamp was used to measure the residual concentration of sodium ion in a sample of sodium DNA which had been dialyzed against a potassium phosphate solution. Instrument readings were taken using the contents of the dialysis bag and compared to those obtained for standard solutions in which the ratio of potassium to sodium ions was approximately one thousand. Note that in this instance high relative concentrations of potassium ion must be included in the standard solution. Less than 0.1% of the original sodium remained within the dialysis bag when the above procedure was employed.

Crystalline 3-aminoacridine was prepared by Dr. Timothy Roy using a standard procedure (80) except for additional purification of the product by vacuum sublimation. Thin layer chromatographic analysis of the purified product



using a variety of solvents and solid supports failed to indicate the presence of any impurities. The spectra of solutions of 3-aminoacridine at pH 11.0 and at pH 2.5 had the same relative absorptivities as those reported by Albert (75). Reported (pH 11.0)  $\lambda_{\text{nm}}^{\text{max}}$  (log  $\epsilon$ ): 237 (4.46), 262 (4.83), 321 (3.35), 337 (3.65), 353 (3.92), 410 (3.79). (pH 2.5) 233 (4.62), 274 (4.65), 349 (4.03), 365 (4.15), 454 (4.10). The melting point agreed with that reported (81): 218°C, uncorr., 223.5°C, corr.

Stock solutions of  $1 \times 10^{-3} \text{M}$  3-aminoacridine were prepared by dissolving 2 mg of the solid in 7 ml of absolute ethanol. The solutions were stored at -5°C in glass. One hundred microliter aliquots of the ethanolic solutions were used for each titration. Periodic tests of the stock solutions using thin layer chromatography showed no observable degradation during the time periods in which the samples were being employed.

Absorptiometric titrations were conducted by successive additions of microliter aliquots of DNA stock solution to 10 ml solutions of 3-aminoacridinium cation. After each addition, the absorbance spectrum between 550 nm and 320 nm of the solution was recorded. To prepare the solutions, 10 ml of supporting electrolyte at a given concentration were measured in a 10 ml volumetric flask. This volume was determined when the liquid was at the same temperature as it was to be during the titrations (e.g., 15.0, 25.0, or 35.0°C). Immediately prior to a titration, 0.1 ml of stock 3-amino-

acridine in ethanol was added to the flask, the contents mixed and then transferred to a 4.00 cm quartz cell (Bolab Inc., Derry, New Hampshire). The cell and its contents were placed in the thermostated compartment of the spectrophotometer (Beckman model DB-GT) and allowed to become thermally equilibrated. The temperature of the instrument's cell compartment was maintained at  $15.0 \pm 0.2^{\circ}\text{C}$ ,  $25.0 \pm 0.05^{\circ}\text{C}$  or  $35.0 \pm 0.2^{\circ}\text{C}$  by circulating thermostated water (Aminco refrigerated water bath) through its external jacket.

The spectrum of the sample was recorded after which an aliquot of stock DNA solution was added. Syringes (Unimetrics, Inc.) were employed to dispense volumes of DNA solution ranging from 4  $\mu\text{l}$  to 100  $\mu\text{l}$  while volumes greater than 100  $\mu\text{l}$  (generally added toward the ends of the titrations) were measured with a 100 microliter micropipette (Centaur Chemical, Danbury, Connecticut). The individual culture tube of the stock DNA solution from which aliquots were being withdrawn for a given titration was maintained at  $25^{\circ}\text{C}$ , regardless of the temperature at which titration was being conducted. After adding the DNA to the 3-aminoacridinium solution in the cell, the solution was mixed by withdrawing about 5 ml of the cell's contents into a serological pipet, then rapidly forcing them back into the cell. This was done three or four times after each addition of DNA.

Concentrations of the stock DNA solutions were determined by measuring the absorbance of a solution containing 50 ml of stock DNA in 10.00 ml of 0.010M supporting electrolyte at pH 5.90 (1.00 cm pathlength). The concentrations of at least ten samples, each taken from separate tubes, were calculated using the molar absorptivity at 260 nm of  $1.413 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$  (75). All glassware was cleaned by immersion into concentrated sulfuric acid saturated with potassium dichromate. Spectrophotometer cells were rinsed with water followed by ethanol and were stored in 50% concentrated hydrochloric acid in ethanol to prevent accumulative absorption of the dye or of DNA to their surfaces.

Solid 7-aminoquinoline was a gift from Dr. D. Jackson, Texas Tech. University, Lubbock, Texas, and was determined to be at least 98% pure according to molar absorptivity values (82-84). Reported (pH 12)  $\lambda_{\text{nm}}^{\text{max}}$  (log  $\epsilon$ ): 240 (4.56), 275s (3.58), 335 (3.68), 346 (3.64). (pH 2.0): 260 (4.44), 285s (3.57), 392 (3.90). The reported pKa of the compound is 6.65 (84). A stock  $3.4 \times 10^{-3} \text{ M}$  solution in absolute ethanol was prepared and 30  $\mu\text{l}$  aliquots added to 8.00 ml of 0.10M  $\text{KH}_2\text{PO}_4$  buffer solution at pH 5.9. The 7-aminoquinolinium solutions were titrated at  $15.0 \pm 0.2^\circ\text{C}$ ,  $25.0 \pm 0.05^\circ\text{C}$ , and  $35.0 \pm 0.2^\circ\text{C}$  using the same equipment and procedures as for the 3-aminoacridinium titrations, vide supra.

### Calculations

The molar absorptivity of the 3-aminoacridinium-DNA complex,  $\bar{\epsilon}_{\text{BHP}}$ , at 368 nm was calculated from absorbance spectra of solutions in which essentially all of the compound was in the bound form. Complete binding ( $\geq 99\%$  of all drug present) was assumed when at least three successive additions of excess DNA to the solution resulted in no change in the total absorbance, corrected for dilution. It was further presumed that, under these conditions, all absorbance at 368 nm was due to the complex and no 3-aminoacridine existed, either as the neutral or monocationic, unbound form. Also, only solutions containing monovalent metals (and tertamethylammonium cation) at low ionicities were used since, for other systems, the large amounts of DNA needed to drive the equilibrium toward products caused serious light scattering and, in some instances, precipitation of DNA salts. Calculated values of  $\bar{\epsilon}_{\text{BHP}}$ , in the presence of each type of electrolyte are presented in Table 1. The averaged value of  $8494 \text{ M}^{-1} \text{ cm}^{-1}$  was used for  $\bar{\epsilon}_{\text{BHP}}$  in the presence of all supporting electrolytes at all concentrations. A similar procedure was used to evaluate the molar absorptivity of the 7-aminoquinolinium-DNA complex,  $\bar{\epsilon}_{\text{AHP}}$ , at its band maximum (405 nm). At pH 5.9 the ratio of neutral to protonated 7-aminoquinoline is 0.178. It was assumed that only the cationic form of the drug binds to DNA. The averaged molar absorptivity of the 7-aminoquinolinium-DNA complex was evaluated

using three simultaneous equations and was found to be  $5332 \text{ M}^{-1} \text{ cm}^{-1}$  at 405 nm.

Concentrations of free 3-aminoacridinium cation, [BH], and bound monocation, [BHP], were calculated from absorptiometric data assuming simple additivity of the absorbances of the two species at the analytical wavelength

$$A = \epsilon_{\text{BH}} \ell [\text{BH}] + \bar{\epsilon}_{\text{BHP}} \ell [\text{BHP}] \quad (2-1)$$

and considering the mass balance expression

$$C_t = [\text{BH}] + [\text{BHP}] \quad (2-2)$$

where

$A$  = the total absorbance of the solution at a given wavelength.

$\epsilon_{\text{BH}}$  = the molar absorptivity of the free species,  
 $1.413 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ .

$\bar{\epsilon}_{\text{BHP}}$  = the molar absorptivity of the bound complex,  
 $8.494 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ .

$\ell$  = pathlength of light in the cell.

$C_t$  = the total concentration of free and bound species.

[BH] = the molar concentration of free 3-aminoacridinium cation.

[BHP] = the molar concentration of bound 3-aminoacridinium-DNA complex.

Solving equation (2-2) for [BH], substituting into equation (2-1), and rearranging yields

$$[\text{BHP}] = \frac{A - \epsilon_{\text{BH}} C_t \ell}{\bar{\epsilon}_{\text{BHP}} \ell - \epsilon_{\text{BH}} \ell} \quad (2-3)$$

Before any DNA has been added, the total absorbance of the solution will be due entirely to free BH and, since BH at this point will be equal to  $C_t$ , the total absorbance before any titration,  $A_o$ , may be expressed as

$$A_o = \epsilon_{\text{BH}} C_t \ell \quad (2-4)$$

Similarly, when all of the compound has been bound, the final absorbance,  $A_\infty$ , is equal to

$$A_\infty = \bar{\epsilon}_{\text{BHP}} C_t \ell \quad (2-5)$$

and for any point in the titration, where the total absorbance is  $A_t$ ,

$$[\text{BHP}] = [(A_t - A_o) / (A_\infty - A_o)] C_t \quad (2-6)$$

and

$$[\text{BH}] = [1 - (A_t - A_o) / (A_\infty - A_o)] C_t \quad (2-7)$$

Since an appreciable amount of the total 7-amino-quinoline exists in the solution in the neutral form at pH 5.9, the equations representing the protolytic equilibrium,

$$K_a = \frac{[\text{H}^+][\text{A}]}{[\text{AH}]} \quad (2-8)$$



the conservation of mass for 7-aminoquinoline,

$$C_t = [A] + [AH] + [AHP], \quad (2-9)$$

and the photometric absorbance at any point in the titration of the small molecule with DNA,

$$A_t = \epsilon_A [A] \ell + \epsilon_{AH} [AH] \ell + \bar{\epsilon}_{AHP} [AHP] \ell \quad (2-10)$$

must be employed to determine the concentrations of the species involved in the binding. Concentrations of drug species are, explicitly,

$$[AHP] = \frac{(\epsilon_A \frac{K_a}{[H^+]} + \epsilon_{AH}) C_t \ell - A_t (1 + K_a/[H^+])}{\{\epsilon_A \frac{K_a}{[H^+]} + \epsilon_{AH} - \bar{\epsilon}_{AHP} (1 + K_a/[H^+])\} \ell} \quad (2-11)$$

$$[AH] = \frac{A_t - \bar{\epsilon}_{AHP} C_t \ell}{\{\epsilon_A \frac{K_a}{[H^+]} + \epsilon_{AH} - \bar{\epsilon}_{AHP} (1 + K_a/[H^+])\} \ell} \quad (2-12)$$

$$[A] = \frac{(A_t - \bar{\epsilon}_{AHP} C_t \ell) K_a [H^+]}{\{\epsilon_A \frac{K_a}{[H^+]} + \epsilon_{AH} - \bar{\epsilon}_{AHP} (1 + \frac{K_a}{[H^+]})\} \ell} \quad (2-13)$$

It was also useful to directly calculate the ratio,

$$\frac{[\text{AHP}]}{[\text{AH}]} = \frac{(\epsilon_A \frac{K_a}{[\text{H}^+]} + \epsilon_{\text{AH}^+}) C_t \ell - A(1 + K_a/[\text{H}^+])}{A_t - \bar{\epsilon}_{\text{AHP}} C_t \ell} \quad (2-14)$$

where

$K_a$  = dissociation constant for the drug or probe,  
 $2.24 \times 10^{-7} \text{ M}^{-1}$ .

$[\text{H}^+]$  = hydronium ion concentration,  $1.26 \times 10^{-6} \text{ M}$ .

$\bar{\epsilon}_{\text{AHP}}$  = molar absorptivity of the bound monocation at  
 the specified analytical wavelength,  $5.332 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$  at 420 nm.

$\epsilon_{\text{AH}}$  = molar absorptivity of the free monocation at  
 the specified analytical wavelength,  $7.943 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$  at 420 nm.

$\epsilon_A$  = molar absorptivity of the free base at the  
 specified analytical wavelength,  $3.47 \times 10^2 \text{ M}^{-1} \text{ cm}^{-1}$  at 392 nm.

$\ell$  = absorption cell path, 4.00 cm.

$A_t$  = the absorbance at any point during the titration  
 at the specified analytical wavelength.

$C_t$  = total concentration of drug or probe.

For each point in a titration the total absorbance was determined along with appropriate constants which were substituted into the necessary relationships to yield the concentrations of free and bound species. Raw data for

all titrations are presented in Tables 2 through 17. Note that volume corrections for the addition of DNA solution to the sample were made in all cases.

The equilibrium association constants for the surface and intercalative modes of binding were calculated for each point in the absorptiometric titrations of the small molecules with DNA, using the relationships developed by A.C. Capomacchia and S.G. Schulman (85).

$$K_s = [\text{BHP}]/[\text{BH}] ([P_{t/3}] - [\text{BHP}]) \quad (2-15)$$

and

$$K_I = [\text{BHP}]/[\text{BH}] ([P_{t/2}] - 2[\text{BHP}])^2 \quad (2-16)$$

where  $K_s$  and  $K_I$  are the apparent association constants for the surface and intercalative modes, respectively, and  $P_t$  is the total molar DNA phosphate concentration. The equations for the binding of 7-aminoquinolinium to DNA are the same except  $[\text{AHP}]$  and  $[\text{AH}]$  are substituted for  $[\text{BHP}]$  and  $[\text{BH}]$ , respectively. The extended Debye-Hückel equation (1-28) was used to calculate activity coefficients at the various ionic strengths, for charged species involved in the association equilibrium.

Apparent association constants were corrected for deviations of molar concentration from activity, by

$$K' = K \frac{\alpha_{\text{BHP}}}{\alpha_{\text{BH}} \alpha_{\text{P}}^n} \quad (2-17)$$

where  $K'$  is the corrected apparent association constant,  $\alpha_{\text{BHP}}$ ,  $\alpha_{\text{BH}}$ , and  $\alpha_{\text{p}}$  are activity coefficients for bound species, free species, and DNA phosphate, respectively. The value of  $n$  is 1 for surface binding and 2 for intercalative binding. For the binding of 7-aminoquinolinium to DNA, the subscripts BHP and BH are replaced with AHP and AH corresponding to bound and free 7-aminoquinolinium, respectively. These, and other calculations, were done using a Litton-Monroe model Beta 326 Scientist computer equipped with a tape cassette drive (Litton-Monroe, Orange, New Jersey). The simultaneous solution of (2-8), (2-9), and (2-10) to obtain concentrations of A, AH, and AHP was accomplished using a program supplied by the computer manufacturer. Programs for specific calculations used here were designed and are presented in Appendix III.

Thermodynamic parameters for the binding of 3-aminoacridinium and 7-aminoquinolinium to DNA were evaluated using equation (1-31). Values of  $\log K$  at 15°C, 25°C, and 35°C for surface and for intercalative binding of each species were determined. These values were then plotted vs.  $1/T$  (K). The slopes of the lines, equal to  $\Delta H^\circ/2.303R$ , were used to calculate the standard enthalpies of binding. Values for the standard entropies of the reactions were evaluated using

$$\Delta S^\circ = (\Delta H^\circ - \Delta G^\circ)/T \quad (2-18)$$

## CHAPTER III

### RESULTS AND DISCUSSION

#### General Spectral and Titration Characteristics

A number of interesting spectral features and titration characteristics are common to all of the systems investigated. Space limitations preclude inclusion of all of the absorptiometric titration spectra, though four which are representative are presented (Figures 3 through 6). These are spectra of titrations done in the presence of the background cation having the lowest charge density,  $\text{Cs}^+$ , and the greatest charge density,  $\text{Mg}^{++}$ , at the highest and lowest concentrations of each. As a rule, the lower the charge and charge density, of the background cation, the less effect it appears to have on the two modes of binding. The highest concentration of the smallest divalent cation represents the other extreme, as it severely inhibits binding processes. The rest of the systems are intermediate between these two. In each of the figures, only 7 curves are drawn for the sake of clarity, though usually between 18 and 22 spectra were recorded for any given titration. For the curves omitted from figures 3 through 6, the absorbances at  $\lambda_{362\text{nm}}$  of the  $\text{max}$

$^1L_b$  bands may be obtained from the appropriate tables in Appendix II.

In all cases, addition of DNA to solutions of 3-aminoacridinium cation resulted in decreases (hypochromisms) in the intensities of both the  $^1L_b$  and  $^1L_a$  bands. It is noteworthy that even small amounts of DNA (< 1 mole DNA per mole of drug) added to 3-aminoacridinium solutions produced only hypochromic shifts. However, addition of small amounts of DNA to other compounds such as 7-aminoquinolinium causes an initial increase in the total intensity (hyperchromism) of the drug's spectrum. Hypochromism subsequently occurs when DNA in excess of about 1 mole per mole of drug is added. Presently, it is not known what factors are responsible for the initial hyperchromism of some species' spectra or why some exhibit initial hyperchromism while others exhibit only hypochromism throughout their whole titration, though significant variations in binding behavior appear highly probable.

It may be seen from Figures 3 through 6 that addition of increasing increments of DNA to 3-aminoacridinium results in red (bathochromic) shifts of both bands of the drug's spectrum. The bathochromism of the  $^1L_b \leftarrow ^1L_a$  and  $^1L_b \leftarrow ^1L_a$  bands are a result of decreases in the energy separations between the ground and excited states. This may be due to a lowering of the  $^1L$  states, a raising of the ground state, or a combination of both. If binding of small molecules to DNA occurs as the reaction proceeds, then it is reasonable



to hypothesize that the cationic drug is moving from a highly polar, high dielectric medium (water) to a less polar, more lipophilic solvent such as the region in, and around, the DNA helix. An environmental change of this type would tend to favor a decrease in charge separation within a molecule and, thus, favor  $\pi \rightarrow \pi^*$  transitions in which the formal positive charge of the heterocyclic ring nitrogen is decreased. Such a case would mean that a lowering of the energies of the excited states is the predominant factor in causing the red shift upon binding if, indeed, such "solvent effect" arguments are valid when considering binding processes. Caution must be exercised in this regard as Capomacchia (69) has found that, for a number of compounds, spectral shifts upon binding to DNA are not in qualitative agreement with shifts observed when the compounds were transferred from polar aqueous solvents to less polar organic solvents. Maximum shifts of the two bands are from 362 nm to 368 nm for the  $^1L_b$  band and from 454 nm to 463 nm for the  $^1L_a$  band. These differences (6 nm and 9 nm, respectively) correspond to decreases in the transition energies of  $450\text{ cm}^{-1}$  (1.29 Kcal/mole dye) and  $428\text{ cm}^{-1}$  (1.23 Kcal/mole dye). The similar magnitudes show that, with excess DNA at low total ionic strength, the two transitions are equally affected from an energetic standpoint in the presence of excess DNA. However, a large number of titrations have substantiated the observation that the  $^1L_b$  moves only slightly, from 362 nm to  $\approx 364$  nm,

over most of the titration and then abruptly shifts from  $\approx 364$  nm to 368 nm while the shift of the  ${}^1L_a$  band is smooth throughout the whole titration. The abrupt shift of the  ${}^1L_b$  takes place at approximately the point in the titration where any additional DNA results in no further decrease in  ${}^1L_b$  band intensity. In fact, this phenomena has been accepted as evidence that all of the protonated 3-aminoacridine has been bound. Moreover, the rapid shift of the higher energy band always occurs significantly beyond the point in the titration where very little change is noted in band intensity or position of the maximum of the longer wavelength  ${}^1L_b$  band. These observations suggest that the environment appears to affect both transitions equally in the presence of excess DNA, but they are affected differently during the course of a titration. A cursory consideration of the data may lead one to postulate that the abrupt shift is due to intercalation of the molecule. This is plausible since internal binding would affect the  ${}^1L_b$  more dramatically than the  ${}^1L_a$ , especially if the charged ring nitrogen remained exterior to the helix. Further consideration, however, reveals substantial evidence, both in this work and in the literature, which shows that intercalation occurs much earlier in the titration and that it is much less abrupt a process than the bathochromic shift considered here would require. The abrupt change might arise from some form of near-neighbor interaction of bound drug molecules (63). During most of the titration, where

the total DNA phosphate to total 3-aminoacridine ratio, P/D, is low, the bound molecules can interact with one another. But as the ratio increases, bound species become isolated from each other to the point of no longer being able to interact. The hypochromic shift is not inconsistent with this proposal as both red and blue shifts are possible upon exciton formation. Another factor to be considered is that as the DNA concentration is increased aggregation of the DNA, itself, may begin to occur. At some critical level, the bound dye may experience a marked decrease in the hydrophilicity of its environment, perhaps as a result of the neighboring polymers excluding water between themselves. Under these circumstances, red shifts would be expected, though why they would be seen only for the  ${}^1L_b$  and not for the  ${}^1L_a$  transition is unanswered.

The onset of the rapid red shift appears to be dependent on the total ionic strength of the medium as well as the type of cations in solution. In the presence of large, diffusely charged, monovalent cesium a distinct bathochromism occurred, even in the highest concentration (0.15M) at the moderate P/D ratio of 65. On the other hand,  $6.3 \times 10^{-4} \text{N Mg}^{++}$  was sufficient to prevent the shift to 368 nm even when phosphate is in excess of drug by a factor of 65. In 0.0025M  $\text{CsH}_2\text{PO}_4$ , a P/D ratio of only 14 results in the spectral shift (in fact, it probably occurs at about P/D = 11). Charge density of the countercations influenced the point at which the abrupt red shift was seen

in a manner parallel to the degree of completion of a titration for a given P/D ratio. The overall spectral behavior of the systems in the presence of the alkaline earths was the same as for the alkalis except the maximum concentrations of  $M^{++}$  that allowed at least 90% binding at  $P/D \leq 100$  was much lower than for alkali metals. Concentrations of group IIa cations in excess of about 0.02N resulted in precipitates forming in the samples. These were, presumably, the metal salts of DNA and appeared long before sufficient polymer was added to appreciably bind the drug.

Throughout this discussion, the absorbances at the band maxima have been assumed to be directly related to the areas under the curves. Since it is the areas, and not peak heights at the maxima which are true measures of transition probabilities, care must be taken in equating peak heights with relative transition probabilities. If the overall geometry of the  ${}^1L_b$  band were to change during the course of a titration, peak heights would not be valid representations of the progress of the titration. In our studies, inspection of the bands showed that their geometries were constant during the titrations, hence, we feel approximating peak heights with peak areas was valid.

In addition, we have assumed that the 0-0 vibronic band maximum of the  ${}^1L_b \leftarrow {}^1A$  transition is coincident with the maximum of the total transition envelope. This may be misleading if the maximum of the envelope arises

from the sums of the intensities of vibronic transitions which are close enough together to appreciably overlap at the maximum wavelength. If the vibronic energies are altered in dissimilar ways during a titration, then the maximal envelope intensity will reflect this dissimilar change along with any real shift in the 0-0 band. It is possible, therefore, that shifts of the  $^1L_b$  envelope maximum may be due to either a disproportionate change in the magnitudes of the band maxima of the 0-0 and 0-1 vibronic bands or a bona fide, pure 0-0 shift.

Variations in the spectral characteristics of the 3-aminoacridinium-DNA systems in the presence of metal phosphates and metal acetates were not due to the different counteranions, as evidenced by the results of titrations using  $KH_2PO_4$  and  $KO_2CCH_3$  as background electrolytes. Results obtained with the two buffer ions were the same in all respects except at very low ionic strengths. Deviations in this region are probably due to lack of sufficient pH control or alterations in the conformation of DNA possibly arising from mild denaturation.

The  $^1L_b$  envelope of the free 3-aminoacridinium cation consists of two distinct vibronic bands having maxima at 348 nm and 362 nm, corresponding to the 0-1 and 0-0 vibronic transitions, respectively. As a titration proceeds, the fine structure of the envelope is lost as a result of the 348 nm peak becoming increasingly less pronounced, to the point of becoming a mere perturbation of the overall envelope. It is



difficult to determine the exact position of the shoulder when the titration is nearly complete, but it appears that its shift is essentially the same as that of the 362 nm band. In cases where the abrupt 364 nm to 368 nm shift of the  $\nu_{00}$  vibronic band of the  $^1L_b$  occurs, there is an accompanying shift of the shoulder maximum from  $\approx 350$  nm to about 354 nm with a slight, but definite increase in the resolution of the two bands. The degree of fine structure of a compound's spectrum provides qualitative information regarding the environment of the species. Loss of fine structure may be rationalized on the basis of increasing the degrees of vibrational freedom of the molecule. If, upon binding, the energy separation between the  $\nu = 0$  and  $\nu = 1$  vibrational levels of the ground state were to be reduced, a coalescing of the two peaks would be anticipated. Such a situation may arise when a molecule moves from an environment in which its vibrational motions are restricted to one in which they are less so. For this to maintain in our systems, the bound species would have to have greater vibrational freedom than the wholly water-solvated free cation. This is feasible considering the strong degree of interaction between water molecules and any dipole in their midst compared to the lipophilic, noninteracting "solvent" of the surface and interior of DNA.

The increase in fine structure which accompanies the dramatic red shift of the 0-0 band of the  $^1L_b$  envelope at high DNA concentrations is not well understood but serves

to corroborate evidence for a dramatic environmental change of bound drug at these very high levels.

To summarize, titrations of 3-aminoacridinium cation with DNA salts in the presence of monovalent and divalent metal ions (including tetramethylammonium ion) of varying concentrations result in hypochromism only. In all cases, both the  $^1L_a$  and  $^1L_b$  envelopes of the free drug's spectrum red shift, at least to some extent, during titration -- the  $^1L_a$  from 454 nm to 463 nm and the  $^1L_b$  from 362 nm to about 364 nm. In the presence of all concentrations of supporting electrolytes having large, monovalent cations, a further, sharp red shift from 364 nm to 368 nm takes place when a P/D ratio of 10 to 15 is reached. The shift may be effected in the presence of smaller alkali metal ions at P/D ratios approaching 70. Regardless of the concentration or charge density of divalent cations, the dramatic  $^1L_b$  shift did not occur. Loss of vibronic fine structure of the  $^1L_b$  envelope was observed in all cases as the titrations progressed, up to the point at which the dramatic bathochromic shift occurred. Concomitant with the rapid shift was a reemergence of the 0-1 vibronic band.

Using relationships developed in the Experimental section, concentrations of free and bound 3-aminoacridinium cation were computed from the total absorbances of solutions which contained varying amounts of DNA. Data for all of the titrations are not included here, though some, which include the extremes of experimental conditions, are presented.



Tables 18 through 21 contain tabulations of [BH] and [BHP] along with  $\log ([BHP]/[BH])$  values for reactions in 0.15M and 0.0025M  $\text{CsH}_2\text{PO}_4$  and in 0.010N and  $6.3 \times 10^{-4}\text{N}$   $\text{Mg}(\text{O}_2\text{CCH}_3)_2$ . In lower concentrations of alkali metals and tetramethylammonium ion, the addition of as little as 5  $\mu\text{l}$  of  $9 \times 10^{-3}\text{M}$  DNA phosphate solution results in a significant degree of binding of the drug. For instance, nearly 10% of the total 3-aminoacridinium present in 0.0025N  $\text{CsH}_2\text{PO}_4$  is bound after addition of only 5.0  $\mu\text{l}$  of DNA. Since volumes of DNA less than 5  $\mu\text{l}$  cannot be measured with much reliability due to its viscosity, the ratio of bound to free drug of 0.1 should be considered as the effective minimum for titrations of this sort. When lower ratios were possible, such as in solutions containing divalent cations or  $\geq 0.010\text{M}$  monocations, random experimental error was sufficiently high to make the data unreliable. As a result, our studies indicate that absorptiometric titrations of small molecules with DNA, under conditions similar to ours, are probably invalid whenever the ratio of bound to total drug is less than 0.1. It should be recalled that our use of 4.00 cm cells maximized sensitivity, thereby allowing minimal increments of DNA solution to be used. Smaller amounts of DNA could be added in 5  $\mu\text{l}$  increments by employing a more dilute stock solution, but the errors arising from other sources would likely be unimproved. At the other titration extreme, ratios of bound to free drug in excess of about 0.95 also result in unacceptable randomness in most

cases. Our procedures are designed to minimize the impact of these sources of imprecision on the calculated association constants.

The maximum extent of total binding which could be achieved varied with the character and concentration of the supporting electrolyte. Table 22 lists the percent of total drug bound to DNA after the final increment of DNA was added in titrations done in various media. The P/D values listed are the maximum that could be obtained due to solubility and pH-control limitations. No effort was made to determine how the drug was bound or what percentage of the total was surface bound compared to intercalatively bound. As discussed previously, for those titrations in which an abrupt bathochromic shift of the  ${}^1L_b$  band was observed, the binding was considered to be complete ( $100 \pm 2\%$ ). The averaged molar absorptivity,  $\bar{\epsilon}_{BHP}$ , determined from these titrations was used to compute concentrations of free and bound species for all systems. In light of the experimental error incurred in evaluating  $\bar{\epsilon}_{BHP}$  and the simple fact that thermodynamic equilibrium demands that there cannot be total binding, percentages greater than about 96% are taken as indicative of complete binding. From Table 22, it may be seen that at least 97% of the total 3-aminoacridinium present is bound in solutions of  $\leq 0.010M$   $MH_2PO_4$ . To attain essentially complete binding in these systems only about 10 to 20 times as much DNA phosphate as total drug need be present (see P/D column of Table 22). As

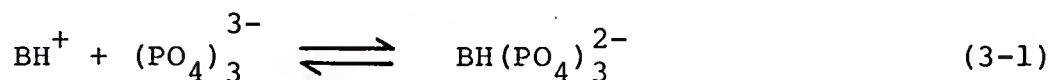
expected, larger and larger ratios of DNA to drug are necessary for complete binding as the concentration of a given electrolyte is increased. Similarly, it becomes increasingly more difficult to shift the equilibrium wholly toward bound product in the order (of electrolyte cations):  $(\text{CH}_3)_4\text{N}^+ \leq \text{Cs}^+ < \text{Rb}^+ < \text{K}^+ < \text{Na}^+$ . A 14-fold excess of DNA was required for 97% binding in 0.0025N  $\text{Cs}^+$ ; 17-fold was needed for 97% binding in 0.010M Cs; while, even with a 65-fold excess of DNA, only 90% of the drug was bound in 0.15M  $\text{CsH}_2\text{PO}_4$ . It was relatively easy to totally bind the 3-aminoacridinium cation in low concentrations of monovalent cations but very difficult to attain greater than 90% binding in even the lowest concentrations of the divalent electrolytes. In  $6.3 \times 10^{-4}\text{N Ba}^{++}$  (the dication having the lowest charge density of those investigated) only 90% of the drug was bound at a P/D ratio of 47 and, for  $\text{Mg}^{++}$  at the same concentration, 90% was bound at P/D = 65. Similarly, in 0.010N  $\text{Ca}^{++}$  and 0.010N  $\text{Mg}^{++}$  the maximum degrees of binding were 77% and 70%, respectively, with P/D ratios of 103 and 104. The choices of the upper limits of total phosphate concentrations reported here were dependent on the solubility of species in the concentrated electrolytes and on pH considerations for the dilute systems. Visible precipitation or erratic spectral behavior were evident in solutions having P/D values greater than those listed in Table 22 for barium and magnesium. The maximum concentration of the divalent electrolytes was set at 0.010N,

since obvious precipitation occurred in solutions of  $0.025N M^{2+}$  well before the titrations were complete (i.e., at 30% to 50% binding). Acetates of the alkaline earths were used instead of their phosphates due to the latter's very low solubilities. Despite dialysis of DNA solutions against pH 5.90 buffer, their pH levels were still apparently close to 7. Thus, addition of moderate volumes of DNA to the very poor acetate buffer systems caused excessive increases in the pH levels of the dilute buffer solutions. Consequently, acetate solutions having more than approximately  $5 \times 10^{-4}M$  DNA phosphate had pH values considerably in excess of 5.90.

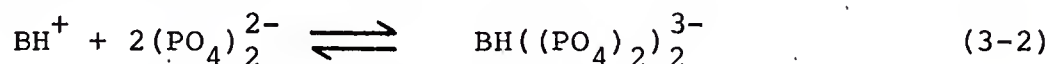
Upon perusing the second column of Table 22, it becomes quickly evident that the extent of binding is not predominately and directly related to ionic strength. Total ionic strengths were computed for the systems having the P/D values listed using equation (1-29) and Program 4 of Appendix III. Notice that while the ionic strengths of  $0.0025M CsH_2PO_4$  and  $6.3 \times 10^{-4}N Ba(O_2CCH_3)_2$  are the same, the binding is complete in the former and only 90% complete in the latter. This shows that the effect of the formal charge on the cations of the electrolyte is greater than predicted on the basis of the simple electrostatic effects incorporated in the limiting Debye-Hückel equation. That the contribution of excess DNA to the total ionic strength is not responsible for these large differences may be seen by comparing  $0.010N$  barium and  $0.010N$  magnesium solutions

which both contain the same amount of DNA and have, therefore, the same total ionic strengths. The extent of binding in the former is 77% while in the latter it is only 70%.

Rapid estimations of the values of  $m$  and  $q$  of equation (1-21) may be obtained by selecting reasonable terms such that  $mq = n$  ( $n$  is total phosphate per drug). It has been shown that (85) in a medium containing 0.002M to 0.005M  $\text{KH}_2\text{PO}_4$  (pH 5.9) the binding of 3-aminoacridinium cation to DNA is best expressed by



for the surface mode, and



for the intercalative mode. In equation (3-1), three DNA phosphates act as a single moiety, reacting with the small molecular cation to form the surface-bound species. In equation (3-2), 2 phosphates act as a single reacting entity with two of these, in turn, interacting with one cation to form the intercalated complex. The values of  $m$  and  $q$  are 3 and 1, respectively, for the first case and 2 and 2 for the second. Substituting these into equation (1-21) yields

$$K_s = [\text{BHP}]/[\text{BH}]([P_t/3] - [\text{BHP}]) \quad (3-3)$$

and

$$K_I = [\text{BHP}] / ([\text{BH}] ([\text{P}_{t/2}] - 2[\text{BHP}])^2) \quad (3-4)$$

$K_S$  and  $K_I$  are the apparent association constants for the surface-bound and intercalatively-bound drug-DNA complexes, respectively. These equations were tested to certify their validity under our experimental conditions. Recall that plots of  $\log([\text{BHP}]/[\text{BH}])$  vs.  $\log([\text{P}_{t/m}] - q[\text{BHP}])$  yield lines whose slopes are equal to  $q$  and whose intercepts are equal to  $\log K$ . Tables 18 through 21 include calculated values of  $\log([\text{BHP}]/[\text{BH}])$ ,  $\log([\text{P}_{t/3}] - [\text{BHP}])$ , and  $\log([\text{P}_{t/2}] - 2[\text{BHP}])$  for representative titrations. Figures 7 and 8 are plots of  $\log([\text{BHP}]/[\text{BH}])$  vs.  $\log([\text{P}_{t/3}] - [\text{BHP}])$  for the reactions in the presence of  $0.0025\text{M Cs}^+$  and  $6.3 \times 10^{-4}\text{N Mg}^{++}$ ; and in  $0.15\text{M Cs}^+$ ,  $0.010\text{N Mg}^{++}$ , and  $0.010\text{M Cs}^+$ , respectively. Figures 9 and 10 are plots of  $\log([\text{BHP}]/[\text{BH}])$  vs.  $\log([\text{P}_{t/2}] - 2[\text{BHP}])$  for the same systems. Three general features are particularly significant. One, the shapes and slopes of the curves are essentially the same whether  $\log([\text{P}_{t/3}] - [\text{BHP}])$  or  $\log([\text{P}_{t/2}] - 2[\text{BHP}])$  is plotted against  $\log([\text{BHP}]/[\text{BH}])$ . (Calculated slopes are tabulated in Table 23.) Only in the latter portions of the titrations done in the more dilute alkali metal electrolytes does this rule begin to break down. Two, except for  $0.15\text{M CsH}_2\text{PO}_4$ , all plots of the data involving the presence of monovalent cations exhibit definite curvature -- the lower the concentration, and further down a series of the periodic chart, the greater the curvature. Conversely, all concentrations of all  $\text{M}^{2+}$  salts yield



essentially straight lines over the whole titration region. Three, in those cases in which there is no curvature throughout the titration range, the slopes of the lines are all nearly 1 and parallel the initial regions of the other titrations.

That the values of  $q$  evaluated from both types of plots are much the same in most media is evidence of the validity of this technique. It appears that going from  $q = 1$  to  $q = 2$  or  $3$  does not substantially affect the values of  $\log([P_{t/m}] - q[BHP])$  except at the extrema of the titrations. With our procedure, however, data very early and very late in a titration need not be relied upon when estimating lines, unlike, for instance, the Scatchard method. For cases where the background electrolyte is especially weak (i.e., low concentrations of cations having low charge density) differences in the slopes of the two plots become appreciable for the later portions of the titrations. For example, the final slopes where  $0.010M$   $Cs^+$  was employed as the background electrolyte are 1.68 and 1.88 for  $q = 2$  and  $q = 1$ , respectively, and for  $0.0025M$   $Cs^+$ , the values are 1.71 and 2.16. Several explanations may be advanced for this lack of agreement, including the fact that the relatively short overall range of the amount of DNA needed to complete the titrations magnifies the error inherent at the extrema. Also, the high degree of curvature makes the determination of the two separate straight line regions more subjective. Perturbations of the reaction

system which are significant only in low ionic strength media (e.g., mild polymer denaturation) would be expected to affect both types of plots in the same manner. Regardless of the sources of the discrepancies, the procedure is still useful, as both plots yield the same integer values of  $q$ . Final selection of  $m$  and  $q$  is made on the basis of the constancy of the computed values of the equilibrium association constant (vide infra).

The presence of at least two distinct linear regions of the plots for lower concentrations of alkali metals strongly indicates two binding stoichiometries. Numerous investigators, as outlined in the Introduction, have shown the presence of two types of binding processes which likely account for the different values of  $q$ . What has not been previously reported is the apparent change in the ratio of phosphate to 3-aminoacridinium cation on going from one type of binding to the other as evidenced by these plots. Also, as the ionic strength is increased, the extent of intercalative binding apparently decreases. This is manifested in Figures 7 through 10 by a progressive trend toward a wholly linear plot as the ionic strength increases. That is, compare the shapes of the curves for systems containing 0.0025M, 0.010M, and 0.15M  $\text{CsH}_2\text{PO}_4$ . Similarly, only one stoichiometry is evident from the plots for  $\text{M}^{2+}$ -containing systems, which are linear throughout their entire titration regions. Slopes of  $1 \pm .1$  for all of these, including that for the reaction in the presence of  $1 \times 10^{-5}\text{N Ba}(\text{O}_2\text{CCH}_3)_2$ ,

indicates that only surface binding occurs in the presence of the divalent metal cations over the titration range investigated.

Previously, it was brought out that, in the presence of low concentrations of monovalent cations as background electrolytes, plots of  $\log([BHP]/[BH])$  vs.  $\log([P_{t/m}] - q[BHP])$  exhibit two regions of linearity. During the early part of a titration the slope of the lines is approximately 1 while in the later region the slope is about 2. As the ionic strength is increased, or as the charge density of the cation of the electrolyte becomes greater, the slope of the later region decreases, eventually becoming equal to about 1. Slopes of the initial portions are essentially unaffected by alterations in the supporting media in which the titrations are conducted.

The ramifications of these relationships may be explored by first defining the binding interaction in terms of conventional metal-ligand chemistry. We shall analogize the cationic 3-aminoacridinium to the metal center and the DNA phosphates to ligands which bind to the center. For clarity, we shall further define two types of ligands, having independent and variable affinities for the cation centers; a weakly bound ligand (corresponding to surface binding) which forms a 1:1 complex, and a more strongly bound ligand (corresponding to intercalative binding) which forms a 1:2 metal:ligand complex. These definitions are based on the values of  $q$  for surface-bound and intercalatively-bound ligand types being 1

and 2, respectively. Finally, we shall assume that the ligands compete for metal binding sites such that if a cation is bound in the surface mode, then it is unavailable for intercalative binding, and vice versa.

During the initial stages of a titration there is a large excess of 3-aminoacridinium over DNA phosphate or, by analogy, an excess of metal over ligand. In such a situation, there would be minimal competition for metal sites and both forms of binding would be expected to occur to their fullest extent, based on their respective association constants. We may view the titration of the cation with DNA ligand as a decrease in the metal center concentration as the process proceeds. Addition of aliquots of DNA serves to limit the number of sites available for binding, causing the more weakly bound surface species to be affected first. At this stage, the strongly bound intercalative species is relatively unaffected. This argument presupposes that the binding affinity of the intercalative mode is substantially greater than that of the surface type. Hence, the first region of the titration reflects surface binding of 3-aminoacridinium to DNA. As the titration progresses, the relative number of available binding sites becomes limited to such an extent that the equilibrium involving the intercalated complex is the one predominantly affected. Now the plot of  $\log([BHP]/[BH])$  vs.  $\log([P_{t/m}] - q[BHP])$  is a reflection of equilibrium concentrations governed by the stronger mode which, in turn, results in the slope of the line being 2

instead of 1. Note that the difference between the relative binding affinities of the two modes must be sufficiently great to allow one to predominate over the other in the initial and final portions of the titrations. On the other hand, if they are too disparate, only one would be seen throughout the experimentally accessible titration range. Increasing the ionic strength of a reaction medium causes electrostatic contributions to the total free energy to become less exothermic for a process in which oppositely charged moieties coalesce. Based on this, decreases in the apparent association constants for both surface and intercalative binding would be anticipated. It is assumed, at this stage of the argument, that changes in electrostatic free energies are the predominant affects accompanying alterations in ionic strength. Extrapolation of the straight lines of Figures 7 through 10 to the vertical axis shows that, as the ionic strength is increased, both  $K_s$  and  $K_1$  do become less positive in agreement with the above prediction. However, such extrapolations may be misleading in the intercalative cases. Recall that, upon altering the ionic strength, the slopes of the initial, surface binding regions of the plots are essentially unchanged and that they form a family of lines. This constancy of slope indicates that there are no changes in the surface binding reaction (aside from equilibrium shifts) as the ionic medium is altered. However, two factors contribute to the decrease in the intercept values of intercalative plots: (1) lower values



of  $\log([BHP]/[BH])$  for any given point along the abscissa as ionic strength is increased, and (2) the decrease in the slopes of the lines as the ionic strength is increased. Two explanations for the change in slope may be advanced. The value of  $q$  may be indeed changing from 2 to 1, for the intercalation reaction, as the ionic strength is increased. If this is true, then either the overall stoichiometry is changing such that  $mq \neq 4$  or, the stoichiometry is remaining constant and  $m$  is becoming equal to 4 while  $q$  is decreasing to 1. A more likely explanation can be advanced on the basis of relative changes of  $K_S$  and  $K_I$ . If  $K_S$  and  $K_I$  do not change in the same manner as the ionic strength is varied, then what we may be observing is an apparent change in  $q$  for intercalative binding when, in reality, the apparent change is due to an overlapping of surface binding manifestations with intercalative binding. Assume that the values of  $m$  and  $q$  remain unchanged for each type of binding, then, the real slopes of the two line segments will remain constant and their point of intersection will be dependent on the ratio of  $K_S$  to  $K_I$ . For example, in one extreme case there is essentially no intercalative binding,  $K_S/K_I$  is very large, and only the line having slope 1 will be observed. At the other extreme,  $K_S/K_I$  would be very small when only intercalative binding occurs and a single line having slope 2 would be seen. If the system is perturbed in a manner which caused  $K_S$  to decrease more rapidly than  $K_I$  the point of intersection of the two lines will reflect the change in



$K_S/K_I$ . In plots such as Figures 7 through 10, this would be manifested as a shift toward the right, or to less negative  $\log([BHP]/[BH])$  values. The total number of sites which are involved in surface and intercalative binding are, of course, directly related to the magnitudes of the equilibrium constants. In other words, an increase in  $K_S/K_I$  indicates that a greater percentage of the total occupied sites are occupied by surface-bound species. The ratio of  $K_S$  to  $K_I$  is seen to increase for the binding reactions between 3-aminoacridinium and DNA in the presence of alkali metal electrolytes as the ionic strength is raised. The following data are representative of  $K_S/K_I \times 10^5$  at ionic strengths of 0.0025, 0.0050, 0.025, 0.050, and 0.10(M), respectively. With  $(CH_3)_4NH_2PO_4$  as supporting electrolyte: 0.44, 0.56, 1.28, 1.83, and 2.9; whereas with  $LiH_2PO_4$  as supporting electrolyte; 0.43, 10.7, 1.2, 5.0, and 10.7, respectively. Excepting the value of  $10.7 \times 10^{-5}$  for 0.005  $LiH_2PO_4$ , the ratios show that as the ionic strength is increased, the total percentage of bound drug which is surface-bound increases. Moreover, for any given ionic strength, the ratio is greater when tetramethylammonium dihydrogen phosphate is the electrolyte than when the lithium salt is present. It is obvious from the decreases in both  $K_S$  and  $K_I$  that increasing the dielectric of the medium does not enhance surface binding relative to intercalative binding. Rather,  $K_S$  does not decrease as rapidly as  $K_I$ . The change in  $K_S/K_I$  may result from specific ion

competition of the electrolyte cations with the drug cations. As the ionic strength increases, the concentration of cations which may directly compete for the DNA phosphate ligands also increases. Moreover, an increase in the effectiveness of competition by the metals for the ligands would be expected as the charge density on the metal increases. Therefore, specific ion interactions may strongly affect surface binding. It is important to note that no evidence from the experiments conducted with the alkali and alkaline earth metals, as counterions, indicated any alterations in surface binding stoichiometry resulting from changes in the medium (aside from the magnitude of  $K_s$ ). That is, all initial slopes of figures such as Figure 7 are equal to 1. Apparent changes in  $q$  for the intercalative region are not likely to be due to intercalation of metal cations because intercalation of the symmetrically charged, highly hydrophilic metal cations is improbable. Specific ion interferences occurring at the surface may, however, affect the overall intercalation of the drug cations.

#### Apparent Association Constants for Surface and Intercalative Binding

Tables 24 through 32 include the apparent association constants for surface binding,  $K_s$ , of 3-aminoacridinium cation to DNA in the presence of various concentrations of background electrolytes. For a given titration, values of  $K_s$  were computed at each data point as discussed in the

Experimental section. By definition, an equilibrium constant is independent of relative and absolute concentrations (specifically, activities) of reactants and products. In fact, this requirement may be used as a test of the validity of an expression postulated to represent an equilibrium situation. Inspection of representative data, included in Tables 18 through 21, reveals that the values of  $K_s$  for points within a specific titration are reasonably constant throughout the initial regions. However, in many cases, notably when low concentrations of alkali metal salts are present, the values calculated from the later points of the titrations are significantly larger than those calculated from the initial portions. For example, in the presence of 0.0025M  $\text{CsH}_2\text{PO}_4$ ,  $K_s$  computed using points from the initial portion of the titration is about  $2 \times 10^5 \text{ M}^{-1}$  while toward the end it is about  $7 \times 10^5 \text{ M}^{-1}$ . It may be recalled, however, that the plots of  $\log ([P_t/m] - q[\text{BHP}])$  for this system, indicate that the value of  $q$  is different for the two regions and, therefore, the binding reactions are different. Thus, when testing various values of  $m$  and  $q$  in search of the pair which results in the most consistent  $K_s$ , it is necessary that  $K_s$  be constant only throughout the region over which it applies. Conversely, upon establishing that specific values of  $m$  and  $q$  are optimal, the test of constancy may be used to define titration regions involving only one binding mode. The region of the titration from which

averaged, reported  $K_s$  values were computed, was the same for all titrations. It has been shown that, as the ionic strength,  $I$ , is decreased, the ratio of bound to free drug at which intercalative binding becomes significant increases. The ratio also increases as the charge density of the counteranion increases. For the most dilute solutions of rubidium, cesium, and tetramethylammonium dihydrogen phosphate, intercalative effects begin to become important when the logarithms of the ratios approach +0.2. In solutions having high ionic strengths, or which contain divalent cations, surface binding predominates even at the highest ratios of bound to free drug. To allow reliable comparisons of calculated surface binding association constants between high and low ionic strength solutions, only data where  $\log ([BHP]/[BH])$  was less than +0.2 were used. The lower limit of the ratio which provides for reliable values of  $K_s$  was estimated to be -0.8. Below -0.8, data often deviated significantly from values obtained above this limit. Using the above guidelines, the averaged values of  $K_s$  listed in Tables 24-32 were computed. It is readily acknowledged that the method of limiting the range of data is somewhat arbitrary and may be eventually replaced with more specific, but as yet unknown, guidelines.

The number of individual data points,  $n$ , used to calculate  $K_s$ , for each titration, is given in the tables. Specific points which were used are indicated by double asterisks (\*\*) in Tables 2 through 12 and 18 through 21.

In most instances,  $n$  was greater than, or equal to 3. Standard deviations from the mean,  $\sigma$ , are also included. Deviations were generally within 5% to 10% of the averaged values of  $K_s$ , which shows that the constancy over the intervals selected is within acceptable limits for these types of systems. As a rule, the lower the concentration of background electrolyte, the greater the standard deviation. Similarly, the lower the charge density of the cation of the supporting electrolyte, the greater the standard deviation. For divalent metal electrolytes the values of  $K_s$  were constant over nearly the entire accessible range. In 0.010N  $\text{Mg}(\text{O}_2\text{CCH}_3)_2$ ,  $K_s$  varied from  $1.32 \times 10^4 \text{ M}^{-1}$  to  $1.21 \times 10^4 \text{ M}^{-1}$  over the entire titration for an average of  $1.27 \times 10^4 \text{ M}^{-1} \pm 5\%$ . In  $6.3 \times 10^{-4}\text{N}$   $\text{Mg}(\text{O}_2\text{CCH}_3)_2$ ,  $K_s$  went from a low of  $4.71 \times 10^4 \text{ M}^{-1}$  to a high of  $5.09 \times 10^4 \text{ M}^{-1}$  (average:  $4.90 \times 10^4 \text{ M}^{-1} \pm 4\%$ ). The essentially invariant apparent equilibrium association constants throughout the entire titrations, along with the single straight line plots of  $\log([\text{BHP}]/[\text{BH}])$  vs.  $\log([\text{P}_t/m] - q[\text{BHP}])$ , is convincing evidence that only the effects of the surface bound species are observed in these systems and that, either intercalative binding is not taking place, or it is being masked. On the other hand, in 0.15M  $\text{C}_2\text{H}_5\text{PO}_4$ ,  $K_s$  varies from an average over the whole titration by  $\pm 25\%$  and by  $\pm 55\%$  in 0.0025M  $\text{C}_2\text{H}_5\text{PO}_4$ . The magnitudes of the standard deviations over the range  $-0.8 \leq \log([\text{BHP}]/[\text{BH}]) \leq 0.2$  are proportional to those given above for the entire



range. The higher deviations at lower ionic strengths may be due to the greater extent of intercalative binding during the initial parts of the titrations at the low ionic strengths. Also, there may be a change in the nature of the surface binding arising from mild denaturation of the DNA. In solutions of low ionic strength, the configuration of the polyanion may be sufficiently altered from that of its native state to affect the surface binding. Addition of even small quantities of DNA to solutions having very low initial ionicities causes large relative changes in their ionic strengths. Therefore, if DNA configuration is dependent on ionic strength and  $K_s$  is dependent on DNA configuration, a substantial variation may be seen over a narrow titration region.

Agreement between apparent surface binding association constants obtained from replicate titrations was generally within  $\pm 10\%$  in the presence of all concentrations of divalent metals and in solutions containing monovalent cations in excess of about 0.010M. In light of the complexity of the systems, errors of this magnitude are considered wholly acceptable. For the more dilute solutions of monovalent electrolytes, agreement between replicate runs was considerably poorer, ranging from  $\pm 35\%$  to  $\pm 3\%$ . It is believed that the lack of precision, under these conditions, stems from uncontrolled perturbations of the configuration of DNA resulting from the low ionic strength. Changes in solution pH may be postulated as an aggravating factor contributing



to imprecision in the poorly buffered, dilute solutions in that Schulman and Capomacchia (85) have shown that pH changes from 5.9 to 7.4 may substantially alter binding of 3-aminoacridinium to DNA. However, this is not likely a major contributor because of the relatively small amount of DNA polyanion added to the solutions. Even if DNA were a strong pH 7 buffer, the amount added would not affect the pH as much as the far greater volumes used for titrations in the presence of dilute alkaline earths where good precision was obtained. Also, at pH 5.9, the  $\text{H}_2\text{PO}_4^-$  anion is a far better buffer at any given concentration than is the acetate anion used with the alkaline earth electrolytes.

The limiting Debye-Hückel law predicts a linear relationship between the logarithms of equilibrium association constants and the square roots of the total ionic strengths of the solutions. Figures 11 through 16 are plots of the log of the apparent surface binding association constant,  $K_s$ , vs. the square root of the total ionic strength,  $I^{1/2}$ . Calculations of  $K_s$  and  $I$ , from which the data for these plots are derived, were based on simple molar concentrations of all species in solution. We have included this data here so that it may be compared with literature data and also to allow comparisons with more refined results presented later. The values of  $K_s$  are the averaged terms reported in Tables 24 through 32 derived from molar concentrations. Similarly, the  $I^{1/2}$  values are

averages of the ionic strength at the beginning and at the end of the regions over which  $K_s$  was obtained and include contributions of all species toward the total ionic strength (all concentrations in molar terms). It is immediately apparent, upon inspection of the figures, that the systems do not obey the limiting Debye-Hückel relationship. Most significantly, all plots exhibit decided curvature over the ionic range investigated. Such non-linearity is not, however, particularly surprising, nor is it necessarily unique to the 3-aminoacridinium-DNA system under consideration since the limiting case is designed only for dilute, ideal solutions of electrolytes. Ideal systems are defined as being infinitely dilute solutions consisting of point charges - ions having no dimensions and engaging in none other than simple electrostatic interactions. Real solutions which approximate the ideal generally consist of simple inorganic ions having high charge densities with total ionic strengths less than 0.01M. Even if our systems were ideal in all respects other than concentration, linearity would be unlikely over the broad ionic strength range studied. The curvature should, if simply a result of deviations from electrostatic ideality, be obviated using corrections included in extended Debye-Hückel equations. These will be considered below.

Reconsidering portions of Figures 11 through 16 in which the total ionic strength is less than 0.01 ( $I^{1/2} < 0.1$ ), we notice that there is approximate linearity, most easily

recognized in the cases of divalent metal electrolytes. Precision of these data may be estimated from their reproducibility at a given ionic strength or from the tendency of data, at various ionic strengths, to form a smooth curve when plotted against  $I^{1/2}$ . Using either of these criteria, it appears that the use of alkaline earth acetates as background electrolytes provides more reproducible results than alkali phosphates. This suggests that pH control may not be the most critical factor in maintaining reproducibility because, for the same ionic strength, the buffer capacity of the phosphates is markedly greater than that of the acetates used for the alkaline earth solutions.

If the conformation of the polyanion is an important factor in surface binding, then perhaps it is maintained more effectively by the divalent cations. Their greater charge and charge density may provide for increased interaction between the polyanion and the metal cations thus generating a disproportionately higher local ionic field around the polymer. Hence, though the bulk ionicities of the two types of solutions are similar, the local electrostatic potential surrounding the polyanion binding sites may be much greater in the presence of the alkaline earths, thereby stabilizing the native DNA. If stabilization in the divalent metal acetate systems were due to acetate rather than phosphate, then the data for  $\text{KO}_2\text{CCH}_3$  as electrolyte would be expected to be more precise than that for  $\text{KH}_2\text{PO}_4$ . Figure 12 shows that such is not the case.

The imprecision at low ionic strengths is sufficiently great as to preclude determining straight lines having slopes agreeing within, say,  $\pm 20\%$  for replicate runs. The large magnitudes of the surface binding constants for our model compound, to DNA, allow nearly optimal conditions of reactant concentrations, compared to many systems reported in the literature. The lack of precision evidenced at ionic strengths less than 0.010N for our investigations indicates that conclusions, based on data obtained at only a single ionic strength below 0.01N, may be suspect.

Figures 11 through 16 reveal a second manner in which these systems disobey the limiting Debye-Hückel law in that, for any given ionic strength, the magnitude of  $K_s$  is a function of the type of background electrolyte. Again, the actual case is not surprising since we are dealing with real ions having finite and differing charge densities. These differences mean that the electrostatic fields around the ions will be dissimilar and, in turn, the dielectric properties of the media will be affected. Extrapolation of plots of  $\log K_s$  vs.  $I^{1/2}$  for dilute solutions of the several different types of electrolytes would, in theory, yield lines intersecting at the ordinate. The y-intercept would correspond to the log of the association constant at zero ionic strength. To extrapolate the lines, in our case, is not feasible due to the imprecision of the data. However, instructive qualitative information may be obtained by

comparing values of  $K_s$  for solutions of intermediate ionic strength. For 0.010M solutions of the following cations, the apparent association constant for the reaction between 3-aminoacridinium cation and DNA decreases in the order:  $(\text{CH}_3)_4\text{N}^+ > \text{Rb}^+ > \text{K}^+ > \text{Na}^+ > (\text{Cs}^+) > \text{Li}^+$ . The order is the same in 0.025M solutions ( $\text{Rb}^+$  was not evaluated at this concentration) and also in 0.050M  $\text{MH}_2\text{PO}_4$ . In 0.10M solutions, the order was  $(\text{CH}_3)_4\text{N}^+ \gg (\text{Cs}^+) > \text{K}^+ > \text{Rb}^+ > \text{Na}^+ > \text{Li}^+$ . In 0.010M electrolytes  $K_s$  ranged from  $17 \times 10^4 \text{ M}^{-1}$  to  $9 \times 10^4 \text{ M}^{-1}$ , in 0.025M electrolytes, from  $11 \times 10^4 \text{ M}^{-1}$  to  $5.4 \times 10^4 \text{ M}^{-1}$ , in 0.050M electrolytes, from  $7.3 \times 10^4 \text{ M}^{-1}$  to  $3.9 \times 10^4 \text{ M}^{-1}$ , and in 0.10M electrolytes, from  $6.1 \times 10^4 \text{ M}^{-1}$  to  $2.9 \times 10^4 \text{ M}^{-1}$ . With the exception of  $\text{Cs}^+$ , the order of the association constants parallels the order of the sizes of the electrolyte cations. Ionic radii for the cations are (86):  $\text{Cs}^+$  (1.68 Å),  $(\text{CH}_3)_4\text{N}^+$  (>1.3 Å),  $\text{Rb}^+$  (1.48 Å),  $\text{K}^+$  (1.33 Å),  $\text{Na}^+$  (0.97 Å), and  $\text{Li}^+$  (0.64 Å). The exact size of the hydrated  $(\text{CH}_3)_4\text{N}^+$  ion is not known but it is likely that it is at least as large as  $\text{Rb}^+$ . It has no available empty orbitals through which any form of dative binding may occur and thereby, provides a means of assessing possible effects of available unfilled metal orbitals on the metal's role in the interaction between the drug and DNA. Qualitatively, the behavior of the system in the presence of the totally saturated tetramethylammonium cation is the same as in the presence of any of the alkali metal cations. Quantitatively, the apparent surface binding association constants are



greatest for the reaction in  $(\text{CH}_3)_4\text{N}^+$  solutions, in accord with its large size and low charge density. There is no evidence that the empty orbitals impart any special properties to the alkali metals in terms of their effects on the binding of 3-AA to DNA.

The seemingly strong suppression of the binding process by cesium ion is not logical in view of the effects of  $(\text{CH}_3)_4\text{N}^+$  and  $\text{Rb}^+$ . Because  $\text{Cs}^+$  is larger than  $\text{Rb}^+$  it would be expected to exert a weaker or, at least, similar effect than rubidium. Despite the fact that  $\text{Cs}^+$  is in the expected order in the 0.10M series, the otherwise anomalous behavior, in its presence, is likely due to some undefined variable such as its original purity. The 20%, by weight, impurity of the solid  $\text{CsOH}$  used to prepare the phosphate, as determined by acid-base titration, was considered to be entirely water of hydration.

At any given ionic strength, based on formal molarities, the alkaline earths more effectively suppress the binding of 3-aminoacridinium to DNA than do the alkali metals. This behavior is not, of course, anticipated on the basis of the limiting Debye-Huckel law but may be partially rationalized with extended equations. The order of the magnitudes of  $\log K_s$  for the binding of 3-AA to DNA in the presence of the alkaline earths, decreases in the order:  $\text{Ba}^{++} > \text{Sr}^{++} > \text{Mg}^{++} > \text{Ca}^{++}$  over the entire range of ionic strength studied (for 0.010N electrolyte,  $\log K_s$  is slightly less with  $\text{Mg}^{++}$  than with  $\text{Ca}^{++}$ ). In decreasing size of



unhydrated cations (86) the order is:  $\text{Ba}^{++} (1.35 \text{ \AA}) > \text{Sr}^{++} (1.13 \text{ \AA}) > \text{Ca}^{++} (0.96 \text{ \AA}) > \text{Mg}^{++} (.65 \text{ \AA})$ . Ion size parameters for the metal ions are 5, 5, 6, and 8, respectively (87). The order:  $\text{Ba}^{++} > \text{Sr}^{++} > \text{Ca}^{++}$  is logical, both in terms of ion size and in terms of ion size parameters. However, calcium more strongly inhibiting the binding of 3-AA to DNA than does  $\text{Mg}^{++}$  is in opposition to charge density and ion size arguments. This reversed order indicates that the effect of the metal ions encompasses more than simple bulk solution electrostatic factors which can be correlated with ion sizes and charge densities. The most apparent additional effect is specific ion competition in which the overall energetics of the competition for phosphate sites must be evaluated.

Figures 17 through 20 are plots of the apparent intercalative association constants,  $K_I$ , against the square root of the ionic strength,  $I^{1/2}$ , for the alkali metals. They are similar to Figures 11 through 16 as they show the relationship between  $\log K_s$  and  $I^{1/2}$  based on conventionally employed, uncorrected molar concentrations. In the presence of the alkali metals, the relationships between the apparent intercalative association constants,  $K_I$ , and the ionic strength were essentially the same as for  $K_s$  and  $I$  discussed above. That is, limiting Debye-Hückel behavior is not obtained over the entire concentration region. Curvature is especially apparent at higher concentrations, and the nature of the background cation affects the degree of

binding. Except in the presence of 0.10M electrolytes, the order of the magnitudes of the intercalative binding constants for 3-aminoacridinium and DNA in the presence of the monovalent cations is the same as for surface binding:  $(\text{CH}_3)_4\text{N}^+ > \text{Rb}^+ > \text{K}^+ > \text{Na}^+ > (\text{Cs}^+) > \text{Li}^+$ . Again, the sequence can be rationalized on the basis of charge density, excepting cesium. In 0.10M solutions, the order is  $(\text{CH}_3)_4\text{N}^+ > \text{Rb}^+ \approx \text{K}^+ > (\text{Cs}^+) > \text{Na}^+ > \text{Li}^+$ . This series differs from the one for  $K_s$  only in the position of  $\text{Cs}^+$  which, again, is probably an experimental artifact. We were unable to observe intercalative binding of 3-aminoacridinium to DNA in the presence of alkaline earth cations at any concentrations. Data were generated and plotted for these systems similar to Figures 17 through 20 but are not included here. The order of the " $K_I$ " values so determined were entirely random with respect to either unhydrated ionic radii or ion size parameters. Possible reasons why the intercalative binding was not seen in these media were presented previously.

To summarize, the relationship between apparent association constants for both surface and intercalative binding of 3-aminoacridinium to DNA over the concentration range of 0.10N to 0.00063M alkali metal background electrolyte and 0.025N to 0.00063N alkaline earth electrolytes do not obey the Debye-Hückel limiting law. In all cases, the plots are non linear and the magnitudes of the association constants are dependent on the nature of the cations. With

the exception of  $\text{Cs}^+$ , the relative effects of the alkali metals parallel their respective charge densities. The same is true for surface binding in the presence of divalent cations, except that  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  are reversed. Plausible comparisons between charge density and relative values of  $K_I$  in the presence of divalent electrolytes cannot be made because of the inability to evaluate intercalative binding in solutions containing the divalent cations. The implications by other investigators that there is a straight-forward relationship between ionic composition of solutions in terms of molarities and binding constants must be considered with reservations.

Association Constants Based on Activities --  
Extended Debye-Hückel Considerations

The procedures outlined above for the determination of binding constants of small molecule to DNA are employed by the majority of investigators in this field. That is, calculations are made with the assumption, either explicit or implied, that molar (or molal) concentrations of species are equal to their activities. Only when activity coefficients are equal to 1 is this assumption strictly valid -- a condition met only by dilute solutions of "well-behaved" systems such as fully ionized, monovalent, inorganic salts. Ideally, evaluation of the relationship between ionic strength and association constants should be based on solutions having total ionic strengths approaching zero, and

including low concentrations of reactants and products. Unfortunately, the conformational instability of DNA in low ionic strength media ( $\leq 0.0050(M)$ ) prevents such investigations. Furthermore, limits of instrumental detectability and the nature of the equilibrium demand moderately high concentrations of the biopolymer. But, while concentrations of background electrolyte and reactants must be higher than desired, a more accurate reflection of the relationship between ionic strength and the association of small molecules to DNA may be made using species' activities as opposed to molar concentrations.

Activities of each electrolyte at the various molar concentrations were estimated by determining the mean activity coefficients of the constituent ions from equation (2-18). Ion size parameters,  $d$ , were obtained from the literature (87) and are included in Table 33. The activities of the electrolytes were used, in turn, in equation (2-18) as the values of  $I$  when estimating the activity coefficients of the species involved in the binding processes. Ion size parameters for BHP, BH, and DNA phosphate were not available and had to be estimated. DNA phosphates were assigned a  $d$  value of  $4 \times 10^{-8}$ , being approximately the same as the value for free  $H_2PO_4^-$ . Free and bound 3-aminoacridinium cation were given  $d$  values of  $3 \times 10^{-8}$  and  $4 \times 10^{-8}$ , respectively. The assignment of ion size parameters for these species is, admittedly, rather

arbitrary as there are few guidelines by which to estimate them. However, it is the effects of varying the background electrolyte which is of principal concern here. So, as long as the values of  $d$  for the species involved in the equilibrium are held constant, relative effects of changing the medium can be estimated though absolute constants may be incorrect. After calculating activity coefficients for reactants and products, the correction factor,  $\xi_s$ , was computed from equation (2-17) where  $\xi_s = \partial_{\text{BHP}} / (\partial_{\text{BH}} \cdot \partial_{\text{P}})$ . Table 33 lists the correction factors for the surface binding along with logarithms of the corrected association constants,  $\log K'_s$ , in the presence of varying concentrations of the electrolytes. For any given initial molarity of alkali metal electrolyte, only minor variations in  $\xi_s$  are observed for the differing types of cations. These small differences are reflections of the minor effect  $d$  has on the correction factor. For instance, values for 0.10M  $\text{LiH}_2\text{PO}_4$  and 0.10M  $\text{CsH}_2\text{PO}_4$  are 4.28 and 4.20, respectively, where  $d$  is  $8 \times 10^{-8}$  and  $2.5 \times 10^{-8}$  for the respective cations. Below about 0.010M electrolyte the correction factor is independent of the character of the monovalent cation. For ionic strengths greater than about 0.01(M), the order of increasing  $\log K'_s$  is the same as for  $\log K_s$ , namely:  $(\text{CH}_3)_4\text{N}^+ > \text{Rb}^+ > \text{K}^+ > \text{Na}^+ > (\text{Cs}^+) > \text{Li}^+$ . (Cesium's position is not the same, but data pertaining to it are probably not reliable, vide supra.) The parallel order for the corrected values with those based on molar



concentrations is expected in light of the relatively minor effect that the ion size parameter has on  $\xi_s$ . Below  $I = 0.010(M)$ , the data is too scattered to make definitive statements. Too, at low ionic strengths, the assumption that DNA phosphates can be treated as if they were species independent of one another becomes especially approximate. Free species may be diluted to any degree whereas, below a given value, further addition of bulk solvent will have little effect on altering the ionic environment of a site on the polymeric surface since its environment is predominantly determined by near-neighbor charges along the polymer and not by charged species in the bulk solvent. Hence, the greater the degree of dilution, the greater the disparity between the calculated ionicity and the true ionic strength local to the binding site.

There is a substantial decrease in the range of  $K'_s$  compared to  $K_s$  values for the range of concentration (activity) studied. At higher ionic strengths the value of  $\xi_s$  is maximal while  $K'_s$  is minimal while at low ionic strength  $\xi_s$  is low with  $\log K_s$  being at its maximum. The product of the two, therefore, tends to decrease the range of  $K'_s$  relative to  $K_s$ . As a result, plots of  $\log K'_s$  vs.  $I^{1/2}$  are more linear as evidenced in Figures 21 and 22. Having corrected for variations in activities of species in solution, and also having presumably eliminated the effects of differing electrolyte ion sizes, theory predicts that plots of  $\log K'_s$  vs.  $I^{1/2}$  would be straight lines which are superimposable.



Obviously, while data for  $I \geq 0.010(M)$  for most of the systems do form reasonably straight lines, they do not superimpose. Moreover, there is still some curvature in the plots which is most pronounced for lithium, becoming less apparent as one goes from  $Na^+$  to  $(CH_3)_4N^+$ . These two features likely result from specific-ion competition by metals for the phosphate sites on the DNA.

Figures 23 and 24 are plots of the corrected surface binding equilibrium association constants,  $K'_S$  vs.  $I^{1/2}$  for reactions in the presence of alkaline earth electrolytes. It can be seen from the figures and from data in Table 33 that the order of the magnitudes of  $K'_S$  in the presence of the alkaline earths is dependent on background electrolyte concentration. This is in contrast to the trends for  $K'_S$  for alkali metal solutions and also, for the uncorrected values of both alkali and alkaline earth electrolytes. The orderings are not random but are due to the unequal slopes of the distinctly straight lines for each type of cation. The lack of superposition of the lines shows that specific ion competition probably is operative.

The above treatment has shown that application of extended Debye-Hückel equations to surface binding data is sufficient to rationalize the nonlinear relationship between  $K_S$  and  $I$ . The remaining deviations are probably due to specific ion effects which will be briefly considered below. Similar attempts to explain the intercalative data are not

so fruitful. The correction factor for the intercalative binding expression,  $\xi_I$ , is essentially a constant, equal to 1, for all ionic strengths investigated (the highest value, for  $I = 0.15(M)$ , is 1.05). Of course, the wide range of  $K_I$  values and the nonlinearity of  $\log K_I$  vs.  $I^{1/2}$  are unaltered by application of the correction for activities. The implications here are uncertain at this time, but it appears that, whatever it is that predominantly affects the intercalative binding, it is not explainable in terms of simple concentrations or ionicities. One possibility is that the intercalative mode is highly susceptible to minor changes in the pitch of the DNA which is, in turn, a function of ionic strength.

#### Evaluation of a Simple Competitive Binding Model

A thorough investigation of specific ion competition between electrolyte metals and 3-aminoacridinium cations, for DNA phosphate sites, would include complex equilibria involving mixed complex systems which are beyond the scope of this work. Such a study would include the selection of probable stoichiometries of metal-DNA, or metal-drug-DNA mixed complexes using procedures similar to those for the drug-DNA systems. These models would then be evaluated to determine the one most probable, on the basis of a "best-fit." The most elementary of these will be briefly considered here.

This system would be one in which there are no mixed complexes and in which the binding stoichiometries of the

metal, and 3-AA, to DNA are the same. It has been shown that 3 DNA phosphates, acting as a single tridentate ligand, bind to each 3-AA, which we shall assume is the same for the metal binding. Considering the 3 phosphates as constituting each site, P, in equations (1-4) and (1-5), we may apply equation (1-7) to our system. Then, the relationship between the observed association constant,  $K'_S$ , for the 3-aminoacridinium-DNA reaction, the actual association constant for drug-DNA binding,  $K''_S$ , and the association constant for the metal-DNA interaction,  $K_M$ , as developed by Passero and Gabbay (64) would be

$$K'_S = K''_S / (1 + K_M M) \quad (3-5)$$

where  $[M]$  is the molar concentration of the electrolyte metal cation. Substituting values of  $K'_S$  and their corresponding  $[M]$  values into equation (3-5) allows for the simultaneous solution of  $K''_S$  and  $K_M$ . Table 34 lists calculated values of  $\log K''_S$  and  $K_M$  for the competitive reactions of alkali and alkaline earth cations and 3-AA for DNA phosphate sites. The concentration ranges of electrolytes listed in Table 34 were selected on the basis of the straight line regions of plots of  $K'_S$  against  $[M]$ . For the simple systems assumed here, there would be a linear relationship between the two parameters because of the first order concentration terms for all species in both equilibrium expressions. Data for lithium as an electrolyte were not included in Table 34 since there was no region of linearity throughout the whole

range of metal ion concentration. This may indicate that the interaction between DNA and  $\text{Li}^+$  is substantially different than between DNA and the other metals. Caution must be advised in drawing conclusions about trends observed in the values of the metal association constants for each series. These values are obtained as the result of small differences between large numbers ( $K'_S$ ) which, in themselves, have large error limits. It is evident, though, that  $K_M$  values for the divalent metals are approximately an order of magnitude greater than the corresponding values for the alkali metals. The logarithm of the equilibrium association constant for the surface binding of 3-aminoacridium cation to DNA, having been corrected for ionic strength, ion sizes of species in solution, and specific ion interactions by alkali metals appears to be  $5.41 \pm 0.10$ . While not as large as for the alkali metal value of  $K''_S$ , the value of  $4.71 \pm 0.08$  for  $K''_S$  in the presence of divalent metal competitors appears reasonable. Having ostensibly corrected for ionic charges, along with the above-mentioned parameters, the values of  $\log K''_S$  for the alkali and alkaline earth series should be equal. That they are substantially different proves that the model needs further refinement. It is logical, though, that the binding stoichiometries of divalent cations and monovalent cations with DNA phosphates would be different.

Thermodynamics of the Binding of 3-aminoacridinium  
and 7-aminoquinolinium to DNA

The standard free energies,  $\Delta G^\circ$ , enthalpies,  $\Delta H^\circ$ , and entropies,  $\Delta S^\circ$ , for the two binding reactions of 3-aminoacridinium and 7-aminoquinolinium to DNA were evaluated from the temperature dependencies of their equilibrium association constants. By comparing the relative magnitudes of  $\Delta H^\circ$  and  $\Delta S^\circ$  for 3-aminoacridinium with those of its benolog, 7-aminoquinolinium, specific effects resulting from variations in the aromatic portion of the molecule may be evaluated. Differences in chemical properties, especially acidities, must be borne in mind when making such comparisons.

The general spectral characteristics of the 3-aminoacridinium-DNA system, along with the regions in the titrations corresponding to surface and intercalative binding and the evaluation of their constants have all been previously discussed for reactions at 25.0°C. Except for small changes in the magnitudes of the equilibrium constants, the systems at 15.0°C and 35.0°C behave in essentially the same manner. These topics will be briefly considered for the reactions between 7-aminoquinolinium and DNA at 25.0°C.

Figure 25 contains representative spectra of the absorptiometric titration of a  $2 \times 10^{-5} \text{M}$  solution of 7-aminoquinolinium with the potassium salt of DNA ( $10^{-2} \text{M}$  in DNA phosphate). The total absorbances of the 7-AMQ solutions

are not as great as for the 3-AA solutions due to the former's lower molar absorptivities (free,  $7940 \text{ M}^{-1}\text{cm}^{-1}$  at 392 nm; bound,  $5332 \text{ M}^{-1}\text{cm}^{-1}$  at 407 nm). Drug concentrations could have been used which would have allowed absorbances greater than 0.4. However, it was decided to use the same formal concentrations of 7-aminoquinoline as 3-aminoacridine. Then, any differences observed in the titration behavior of the two could not be due to differences in their concentrations. Also, the lower binding affinity of 7-aminoquinolinium for DNA makes it desirable to keep its concentration at a minimum to allow maximum ratios of DNA to drug without introducing complicating concentration problems.

The visible spectra of both the free and bound 7-aminoquinolinium cation consist of only one envelope, having no fine structure, unlike the spectra of the 3-aminoacridinium species which have well differentiated  $^1\text{L}_a$  and  $^1\text{L}_b$  bands. Presumably, substitution of the amino group in the  $\beta$  position of the molecule results in the  $^1\text{L}_b$  transition having increased probability over the  $^1\text{L}_a$ . Thus, the observed spectra consist predominantly of the  $^1\text{L}_b \leftarrow ^1\text{A}$  transition. The  $^1\text{L}_a$  band may be buried beneath the  $^1\text{L}_b$  though no attempts were made to ascertain its position. The lack of distinct  $^1\text{L}_a$  and  $^1\text{L}_b$  bands and the absence of fine structure in the spectra of either the free or bound species preclude discussions concerning possible molecular orientations upon binding. There is no evidence to indicate,



however, that 7-aminoquinolinium does not interact with DNA in essentially the same manner as does 3-aminoacridinium. Similar reaction stoichiometries and values of  $m$  and  $q$  in equation (1-21) are in agreement with this presumption. Significant shifts in the baseline of the spectra occur with the addition of moderate to large amounts of DNA. Figure 25B is a reproduction of a titration spectrum in which it may be seen that the absorbance at wavelengths greater than 475 nm increases by approximately 0.03 absorbance units upon addition of 1400  $\mu$ l of  $10^{-2}$ M DNA phosphate to 8.04 ml of solution (resultant P/D = 105). Magnitudes of baseline shifts for 7-aminoquinolinium titrations are particularly significant in view of the relatively small total changes in absorbance, at the analytical wavelength, throughout the titrations. Reverse titrations in which stock DNA solution was used to titrate pH 5.90 buffer in a 4.00 cm cell showed that an increase in the baseline absorbance of about 0.03 units may be anticipated from light scattering arising from the presence of relatively high concentrations of DNA polymer. Correcting the absorbances at 500 nm of each individual spectra to zero results in absorptiometric titration spectra similar to the one shown in Figure 25A. Here it becomes evident that what appeared to be an isobestic point at  $\approx$  420 nm was, in fact, an artifact and, thus, the system may involve more than two species which are in equilibrium. It is also evident that, even after addition of 1400  $\mu$ l of DNA solution to the 8.04 ml of

drug, ( $P/D = 105$ ) the titration was not complete. Adding greater amounts of DNA is counterproductive because of increasingly severe light scattering and the dilution of the sample. Baseline absorbance at the analytical wavelength was determined at the point of intersection of a line drawn between the absorbances at 475 nm and 325 nm, and a perpendicular at the analytical wavelength. The analytical wavelength was that of the band maximum for the total solution.

The band maximum for free, protonated 7-aminoquinolinium is at 392 nm. As DNA is added to the drug, there is both a decrease in the intensity of the band and a red shift of its maximum. The hypochromism and bathochromism are both qualitatively similar to those seen in the titrations of 3-aminoacridinium. However, the 7-AMQ red shift indicates a significantly greater energy difference between the free and bound 7-aminoquinolinium compared to the difference between free and bound 3-aminoacridinium. In the presence of a 100-fold excess of DNA phosphate to drug, the band maximum for bound 7-aminoquinolinium appears at 407 nm corresponding to a difference in the transition energies between the ground and excited states of the free vs. the bound complexes of  $940 \text{ cm}^{-1}$  (2.70 Kcal/mole 7-AMQ). Recall that the transition energy difference for free and bound 3-aminoacridinium amounted to only about 1.3 Kcal/mole 3-AA.

There were no titrations of 7-aminoquinolinium in which an abrupt shift in the spectra, similar to that seen in the

$^1L_b$  band of the 3-aminoacridinium system, was observed.

The absence of a dramatic shift at DNA phosphate levels in excess of those causing shifts in the 3-aminoacridinium systems shows that shifts in the latter instances were due to something other than simple changes in the bulk solvent's composition. It is not known if greater concentrations of DNA would have resulted in dramatic shifts in the 7-aminoquinolinium spectra.

Figures 26 and 27 are plots of  $\log([AHP]/[AH])$  vs.  $\log([P_t/3] - [AHP])$  and  $\log([P_t/2] - 2[AHP])$ , respectively for the titrations of 7-aminoquinolinium with DNA at 15.0°C, 25.0°C, and 35.0°C. In all instances, two distinct regions of linearity are seen, indicative of two separate modes of binding. In Figure 26 the averaged slopes of the initial portions of the curves, corresponding to the surface binding process, is  $1.03 \pm 0.13$ . This value is nearly the same as for 3-aminoacridinium and shows that equation (2-15) also applies to 7-aminoquinolinium surface binding. The averaged values for the same region of Figure 27 is  $1.02 \pm 0.12$ . The value for  $q$  for the intercalative binding, obtained from the slopes of the later regions of the lines in Figure 27, is  $1.64 \pm 0.09$  (from Figure 26,  $q = 1.8 \pm 0.4$ ). These are somewhat ambiguous in terms of the actual integral value of  $q$  but there is little doubt that it is greater than 1 and is presumed to be less than 3, based on the lack of constancy of the computed values of  $K_1$  when  $q = 3$ . Because of the relatively weak binding of 7-aminoquinolinium to DNA, it was

not possible to titrate the system in a region in which the surface binding did not overlap intercalative binding. As a result, the later points in Figures 26 and 27 probably include some contributions from surface binding which would cause the slopes to be less than 2. On the bases of best-fit analyses and the similarity of the binding with that of 3-aminoacridinium, values of  $m = q = 2$  were selected for the intercalative mode of 7-aminoquinolinium binding to DNA.

Table 35 includes the calculated apparent association constants for the surface and intercalative binding of 3-aminoacridinium and 7-aminoquinolinium to DNA at 15.0°C, 25.0°C, and 35.0°C. Constants were computed using the estimated activities of the species (equations (1-30) and (2-17)). An ion size parameter equal to  $3.5 \times 10^{-8}$  was used for the free drug cations and bound complexes. For any given temperature, the apparent surface binding association constant of 7-aminoquinolinium is approximately an order of magnitude lower than for 3-aminoacridinium while the constants for the intercalative process are about two orders of magnitude lower for the former. Magnitudes of  $K_s$  and  $K_1$  for both compounds decrease with increasing temperature indicating a negative free energy for the binding process. Figures 28 and 29 are plots of  $\log K'_s$  vs.  $1/T$  (°K) for 3-aminoacridinium and 7-aminoquinolinium, respectively, and Figures 30 and 31 are similar plots of  $\log K'_s$  vs.  $1/T$  (°K). With the possible exception of Figure 31, the relationship

between  $\log K'$  and  $1/T$  is linear as predicted by the van't Hoff relationship (equation (1-31)). Values of the standard enthalpy for the reactions, included in Table 36, were calculated from the slopes of the lines of Figures 28 through 31. Standard entropies of binding were computed from values of  $\Delta H^\circ$  and  $\Delta G^\circ$  at  $298^\circ\text{K}$ . The standard state upon which the thermodynamic parameters are based is related to activities of the species estimated from their molar concentrations using equation (1-30). While the total free energy of binding of 3-aminoacridinium is more negative than for 7-aminoquinolinium ( $\Delta G^\circ = -7.35$  Kcal/mole 3-AA vs.  $-6.07$  Kcal/mole 7-AMQ) the standard enthalpy change or heat of reaction involving 3-aminoacridinium is less than that involving 7-aminoquinolinium ( $\Delta H^\circ = -4.90$  Kcal/mole 3-AA vs.  $-5.13$  Kcal/mole 7-AMQ). Enthalpy is predominantly a measure of the overall energy changes resulting from the disruption of bonds associated with reactants and the formation of new bonds among product species. Considered in this vein, it is evident that the greater binding affinity of 3-aminoacridinium over 7-aminoquinolinium for DNA cannot be attributed to electrostatic bonding energies because, based solely on  $\Delta H^\circ$ , the binding of 7-AMQ to DNA would be greater than that of 3-AA. Two types of electrostatic interactions are probably most significant here: the ion-dipole forces between drug cations and water molecules of the solvent, and the ion-ion interactions of the charged ring nitrogens with the DNA phosphate anions. Ion-dipole forces



between solvent molecules and the phosphate anions would be less important than the above two. The two drug cations are similar in terms of the structural characteristics which would affect the attractive forces between them and water molecules. Both have hydrogen-bond acceptor amino groups and protonated hydrogen-bond ring nitrogens. Thus, to a first approximation, the total energy necessary to disrupt the ion-dipole interactions between water and 3-amino-acridinium cations would be expected to be about the same as that for water and 7-aminoquinolinium. More important, though, is a consideration of the relative acidities and basicities of the groups. The extent of hydrogen bonding between the solvent and a hydrogen-bond acceptor is related to the acceptor's basicity while the extent between the solvent and a hydrogen-bond donor can be correlated with the donor's acidity. The protonated heterocyclic ring nitrogens are hydrogen-bond donors. Therefore, 7-aminoquinoline ( $pK = 6.65$ ), being a stronger acid than 3-aminoacridine ( $pK = 8.04$ ), will form stronger hydrogen bonds with water making the  $\Delta H^\circ$  for its binding reaction less positive than that of 3-AA. Likewise, the basicity of the substituted amino group of 7-aminoquinoline is somewhat greater ( $pK = 0$ ) than the amino group on 3-aminoacridinium ( $pK_a = -1.5$ ), meaning the hydrogen-bond acceptor of the former would interact more strongly with water than the latter, thus, generating a less negative  $\Delta H^\circ$  for reactions involving 7-AMQ as a reactant. The above factors tend to make the change in



enthalpy for the reaction of 7-aminoquinolinium with DNA less negative than that of 3-aminoacridinium with DNA. It is clear that simple arguments based solely on ion-dipole energies of the reactant cations are not sufficient, in themselves, to explain the more negative  $\Delta H^\circ$  for the 3-AA system.

The amount of energy evolved upon formation of the electrostatic ion-ion bonds between the cationic drug molecules and the anionic phosphates of the DNA may explain the slightly more negative  $\Delta H^\circ$  for 7-aminoquinolinium binding over that for 3-aminoacridinium. The cation having the greater charge density at the heterocyclic nitrogen would bind more strongly. 7-aminoquinolinium, being the stronger acid, would generate the stronger electrostatic interaction of the two, in agreement with the experimentally observed values. The effect of the third ring of 3-aminoacridine as a steric barrier to hydrogen bonding of the ring nitrogen would serve to make  $\Delta H^\circ$  for the reaction involving 3-AA more negative than for 7-AMQ, but this effect would likely be small.

Since the binding sites are presumed to be identical for the two cations, the energies involved in disrupting bonds associated with the solvent and the DNA binding sites would be the same. Likewise, enthalpic contributions arising from the formation of the solvent cages surrounding the bound species would be expected to be similar if the binding geometries of the two are alike.

Intercalation of 7-aminoquinolinium and 3-aminoacridinium may occur by their "slipping" between DNA base pairs. Such a mechanism would allow for interactions between the aromatic base pairs and the ring systems of the cations and thus the 3-AA, having a larger aromatic surface area, might be expected to interact more strongly.

Changes in the standard enthalpies for the intercalative binding processes of the two cations to DNA are more negative than for the surface binding, but are qualitatively similar in that  $\Delta H^\circ$  for 7-aminoquinolinium is slightly more negative (-15.1 Kcal/mole) than for 3-aminoacridinium (-13.7 Kcal/mole). The absolute magnitude of  $\Delta H^\circ_s$  for 7-AMQ is 1.06 times greater than  $\Delta H^\circ_s$  for 3-AA while the absolute magnitude of  $\Delta H^\circ_I$  for 7-AMQ is 1.10 times greater than  $\Delta H^\circ_I$  for 3-AA. These similar ratios show that in terms of the enthalpic contributions to the free energy of binding, intercalation of 3-aminoacridinium and 7-aminoquinolinium are the same. That is, the larger, more lipophilic aromatic portion of 3-AA does not impart any particular advantage to the compound in terms of the enthalpy of the intercalation process.

The entropy of surface binding of 3-aminoacridinium is considerably more positive than for the similar process with 7-AMQ. For 3-AA,  $T\Delta S^\circ$  at 298°K equals 2.45 Kcal/mole and for 7-AMQ,  $T\Delta S^\circ$  at 298°K is 0.94 Kcal/mole. In fact, it is this relatively large difference in entropy which obviates the enthalpic advantage 7-AMQ has over 3-AA, resulting in the more negative free energy of binding of 3-aminoacridinium.

Entropy change is a measure of the extent to which the randomness of a system is altered during the course of a reaction, being positive when the final state is less ordered than the initial state. In terms of the stoichiometries of reactants and products,  $\Delta S^\circ$  for the two would be expected to be the same. Obviously, the solvent and the solute's effect on its structure are of great importance in the present instance. The role of solvent structure may be understood by envisaging the bulk solvent as having a relatively random, fluid structure. Dispersed throughout the solvent are the organic cations, 3-AA and 7-AMQ, which both disrupt the bulk solvent structure by their presence. But the tricyclic 3-aminoacridinium, being more lipophilic and larger in size, will demand a greater extent of solvent structuring to accommodate it. We have already considered the effects that the polar regions of the cations would have on the water via hydrogen bonding. Therefore, since the DNA sites of the two species are presumed to be the same, the binding of 3-aminoacridinium will result in a more positive entropy change as the cation is "removed" from the aqueous solvent. This would be true even if surface binding effected only partial removal of the cationic species from the water.

Large error limits for the values of  $\Delta S^\circ$  for the intercalative binding of the two cations to DNA mandate caution in comparing them, though it appears that  $\Delta S^\circ$  for the binding of 7-aminoacridinium is considerably less positive than for

3-aminoacridinium. This relationship is in parallel with the surface binding  $\Delta S^{\circ}$  values and may be due to the same factors as discussed above. The entropy changes given in Table 36 are per mole of cation but, since the surface and intercalative modes involve different ratios of DNA phosphate to cation, comparisons of the entropies of the two processes can be misleading. However,  $\Delta S^{\circ}$  terms for surface binding are significantly positive whereas for the intercalative mode they are, within experimental error, nearly zero or less than zero. Intercalative binding entropy changes include a positive contribution arising from the release of water molecules as the cations are removed from the bulk solvent. These would likely be about the same as the  $\Delta S^{\circ}$  terms for surface binding meaning that the entropy associated with the insertion of a 3-AA cation already on the surface of the polymer would be approximately  $-10.4$   $(-2.2 - 8.2)$  e.u. or  $-3.10$  Kcal/mole at  $298^{\circ}\text{K}$ . For 7-AMQ the value would be about  $-17$  e.u.  $(-14 - 3)$  or  $-5.07$  Kcal/mole at  $298^{\circ}\text{K}$ . These negentropic terms indicate that it is the highly negative enthalpies of the intercalative reactions which result in the overall negative free energy of binding. Surface bound cations will apparently spontaneously bind intercalatively due to the small enthalpic advantage afforded the latter process. It appears that, while the enthalpy arising from intercalation is decisive, it is only slightly more negative for 3-aminoacridinium than for 7-aminoquinolinium.

Earlier, we discussed the binding reactions in terms of ligand-metal models. Thus, it is instructive to continue the analogy to specifically include the chelate effect. The well known chelate effect (88) refers to the more negative free energy of formation of polycordinate metal complexes formed from polydentate chelates over similar complexes formed from monodentate ligands of chelates having fewer coordination sites. For example,  $\log \beta_4$  for  $\text{Cu}(\text{NH}_3)_4^{2+}$  is 11.9 while  $\log \beta_2$  for  $\text{Cu}(\text{H}_2\text{NCH}_2\text{CH}_2\text{NH}_2)_2^{2+}$  is 20.0 (89) in which the former is formed from 4 monodentate ammonia ligands and the latter from 2 bidentate ethylenediamine chelates. The chelate effect for non-transition metals is almost entirely an entropy effect (90). For transition metals, greater crystal field stabilization of chelates over monodentate complexes introduces an enthalpy contribution (91). The more positive entropy for chelate binding means, in terms of probability, that it is easier to form an aggregate from a few molecules than from many molecules. The three DNA phosphates involved in surface binding could conceivably act as three monodentate ligands, a monodentate ligand and a bidentate chelate, or as a single tridentate chelate. The case in which the phosphates act as a single chelate gives the most consistent calculated values of  $K_s$  and is also the one which would be expected based on the chelate effect. On the other hand, the 4 phosphates involved in intercalative binding could react as 4 monodentates; 1 monodentate and 1 tridentate; 2 monodentates and 1 bidentate; 2 bidentates; or

as 1 tetradentate ligand. Clearly, the chelate effect predicts that the tetradentate ligand would generate the most positive entropy. However, the use of a  $(\text{PO}_4)_4^{4-}$  term in the equilibrium association expression yields values of  $K_I$  which are not nearly as consistent throughout the intercalative titration region as those when  $2(\text{PO}_4)_2^{2-}$  is employed. Thus, an antichelate effect is seen for the intercalative process in which the greater  $\Delta H^\circ$  for the 2-bidentate-ligand mechanism appears to be sufficient to compensate for any positive entropy gain which might arise from a reaction involving a single tetradentate chelate.

When discussing the forces which account for the behavior of polymers and biologically important systems, such as lipid membranes in aqueous media, the "hydrophobic bond" is often invoked. The avoidance here of any reference to this term as an aid to explaining the energetics of the binding reactions has been intentional. In most cases, "hydrophobic forces" are merely manifestations of well established fundamental forces arising from energies of adhesion and cohesion and from entropic factors. It perhaps could be argued that the "driving force" for the intercalative binding of the compounds to DNA is the formation of hydrophobic bonds between the aromatic rings of the cations and the aromatic portions of the DNA bases. But as Hildebrand (92) has pointed out, it is rare where the energy involved in the interaction of two organic species in an aqueous system is any greater than it is in a corresponding non-aqueous environment. For example, the alkyl chains in soap micelles

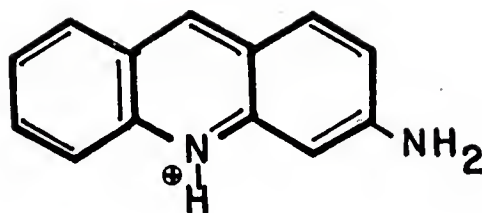


are not bonded together by a phobia for water -- they are just as adhesive in the absence of water. In other words, there is not necessarily any phobic response of the species toward water, but rather the water molecules would "prefer" to be hydrogen bonded together rather than separate to admit the immiscible molecules.

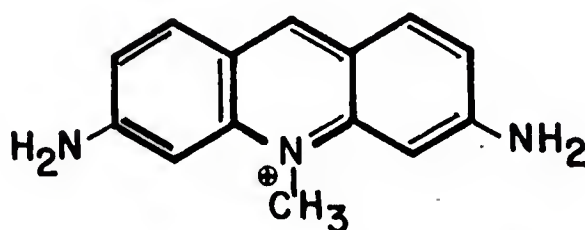
APPENDIX I

FIGURES

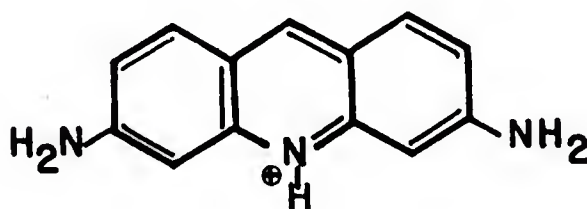
Figure 1. The structures of selected substituted  
acridines and related compounds  
(Structures I through XVI)



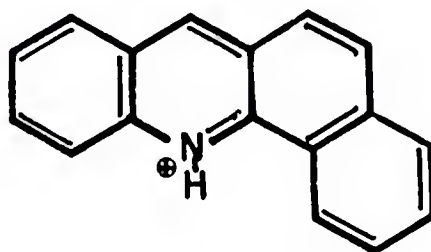
I. 3-AMINOACRIDINIUM CATION (3-AA)



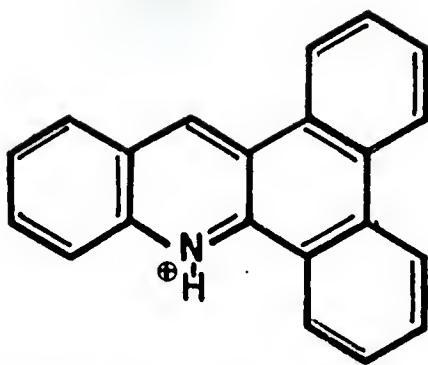
II. 3,6-DIAMINO-10-METHYLACRIDINIUM CATION (ACRIFLAVIN)



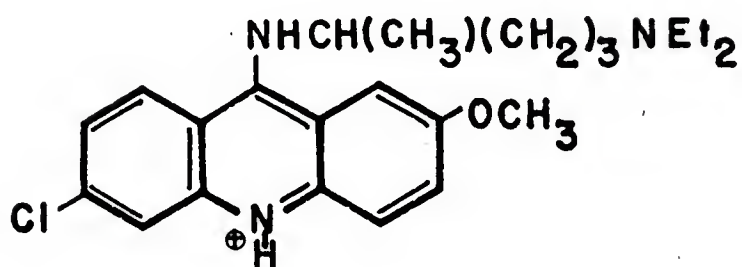
III. 3,6-DIAMINOACRIDINIUM CATION (PROFLAVINE)



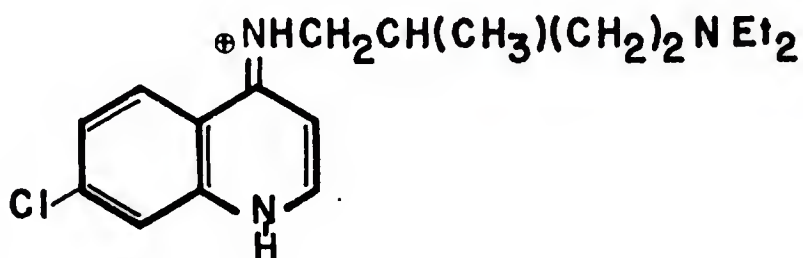
IV. BENZ [c]ACRIDINIUM CATION



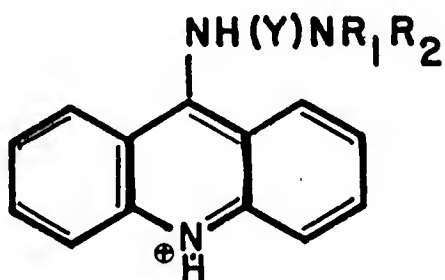
V. DIBENZ [6,6] ACRIDINIUM CATION



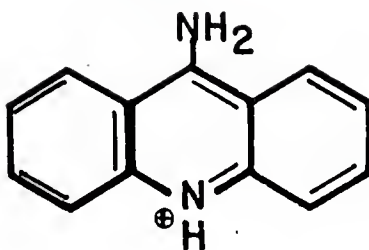
VI. QUINACRINE CATION (ATEBRIN, MEPACRIN)



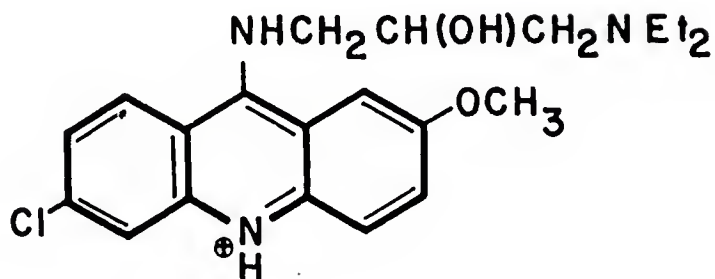
VII. CHLOROQUIN CATION



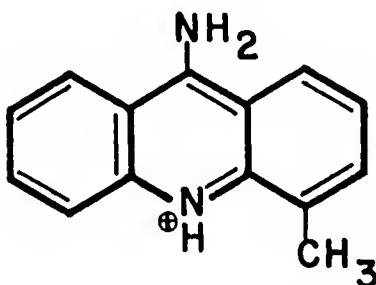
VIII. GENERAL STRUCTURE OF SOME  
ANTIMALARIALS



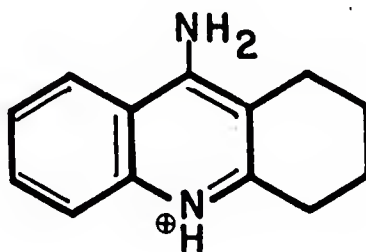
IX. 9-AMINOACRIDINIUM CATION



X. ACRANIL CATION

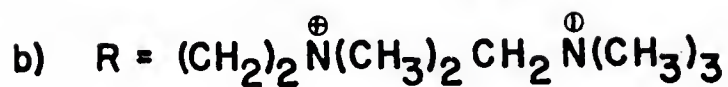
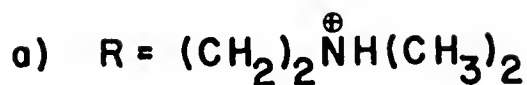
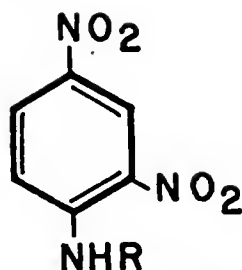


XI. NEOMONACRIN CATION

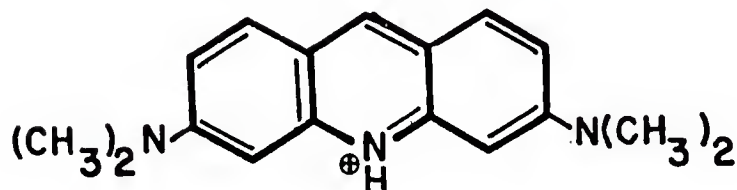


XII. 9-AMINO-1,2,3,4-TETRAHYDROACRIDINIUM CATION

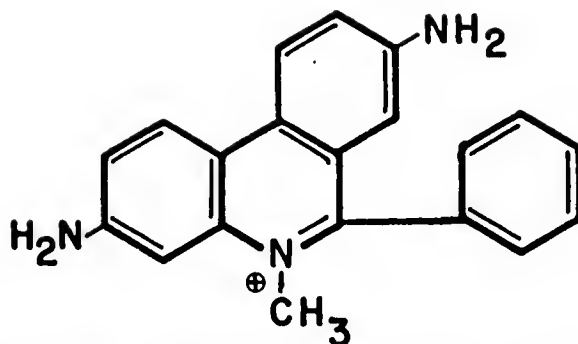




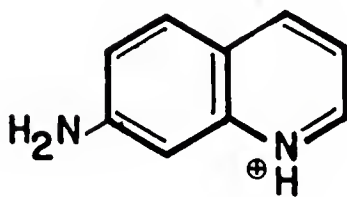
XIII. REPORTER MOLECULES FROM REFERENCE  
(31)



XIV. 3,6-DIMETHYLAMINOACRIDINIUM  
CATION (ACRIDINE ORANGE)



XV. ETHIDIUM BROMIDE CATION



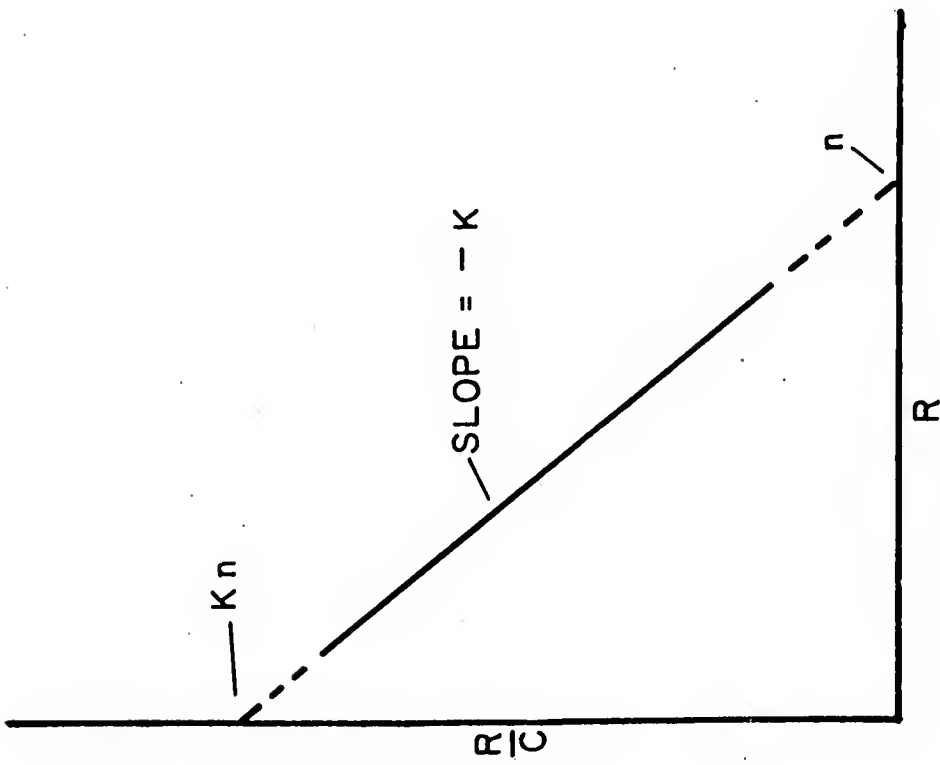
XVI. 7-AMINOQUINOLINIUM CATION (7-AMQ)

Figure 2. General form of Scatchard plots: R/C vs. R for one and two classes of binding sites

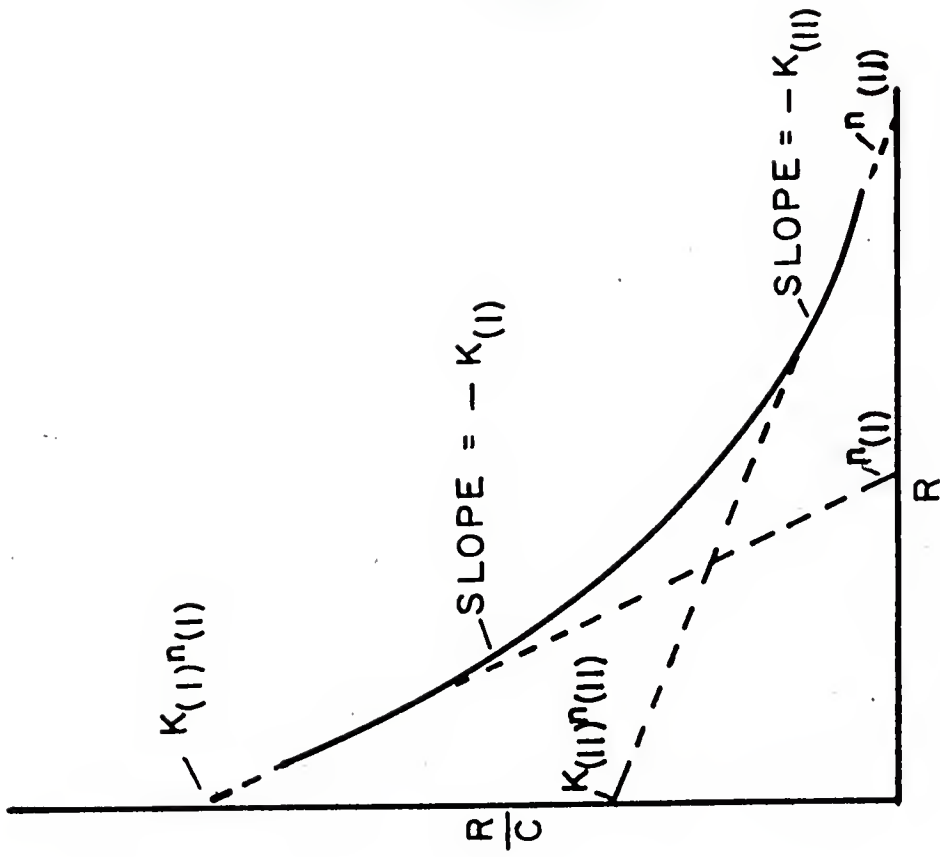
A One class of binding site ( $K, n$ ).

B Two classes of binding sites

( $K_I, n_I; K_{II}, n_{II}$ ) where  $K_I \gg K_{II}$



(a)



(b)

Figure 3. Absorptiometric titration of 3-aminoacridinium cation with DNA, cesium salt, pH 5.90, 25.0°C. Background electrolyte:  $\text{C}_s\text{H}_2\text{PO}_4$ , initial concentration, 0.15M

- a  $\text{P/D} = --$  ( $[\text{P}] = 0$ )
- b  $\text{P/D} = 2.89$
- c  $\text{P/D} = 5.97$
- d  $\text{P/D} = 9.65$
- e  $\text{P/D} = 17.0$
- f  $\text{P/D} = 26.7$
- g  $\text{P/D} = 65.3$

P/D is the ratio of total DNA phosphate to total 3-aminoacridinium cation,  $\text{D} = 1.35 \times 10^{-7}$  moles.

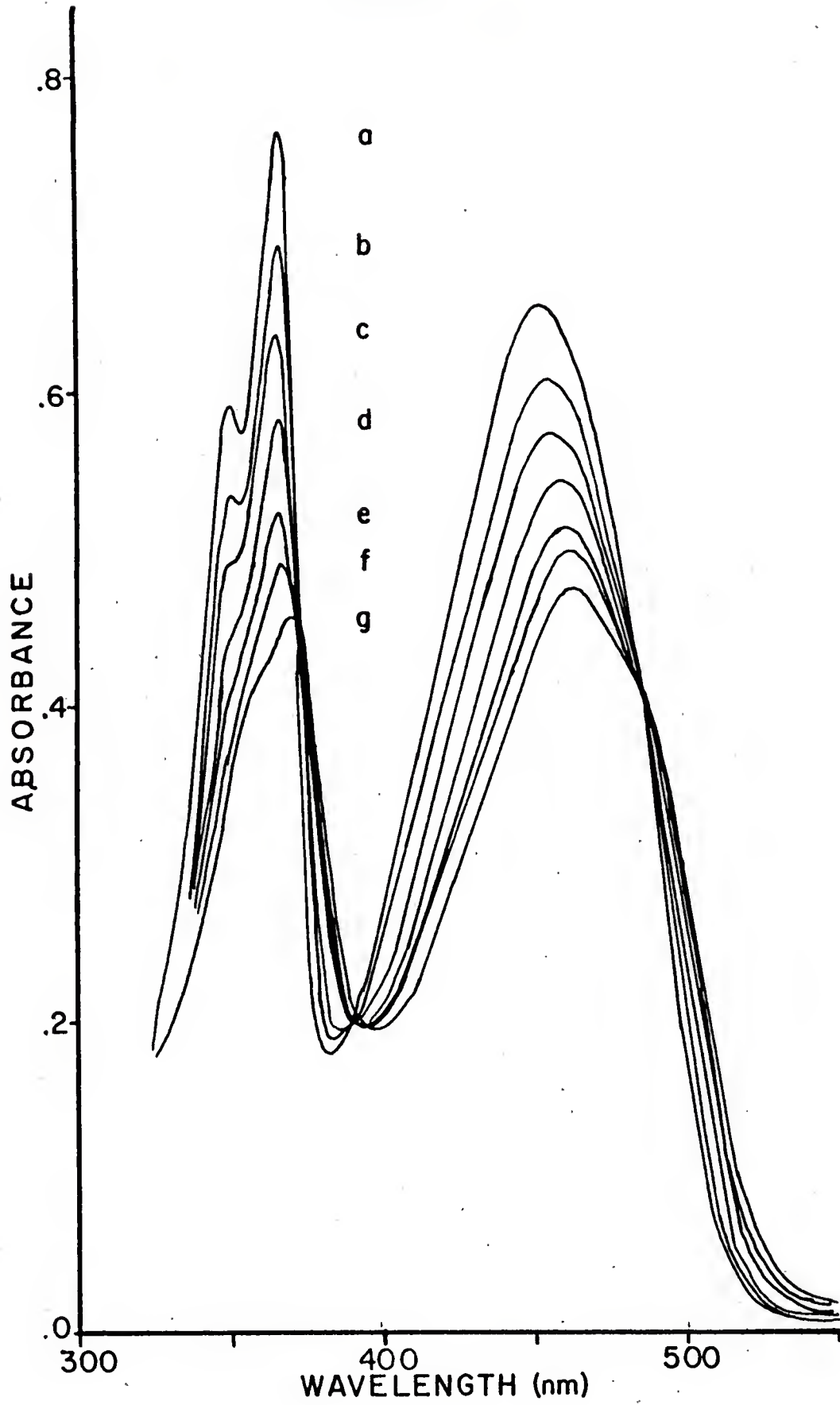




Figure 4. Absorptiometric titration of 3-aminoacridinium cation with DNA, cesium salt, pH 5.90, 25.0°C. Background electrolyte:  $\text{C}_5\text{H}_2\text{PO}_4$ , initial concentration, 0.0025M

- a  $\text{P/D} = \text{--} ([\text{P}] = 0)$
- b  $\text{P/D} = 0.652$
- c  $\text{P/D} = 1.63$
- d  $\text{P/D} = 2.94$
- e  $\text{P/D} = 4.57$
- f  $\text{P/D} = 5.87$
- g  $\text{P/D} = 13.7$

P/D is the ratio of total DNA phosphate to total 3-aminoacridinium cation,  $\text{D} = 1.33 \times 10^{-7}$  moles.

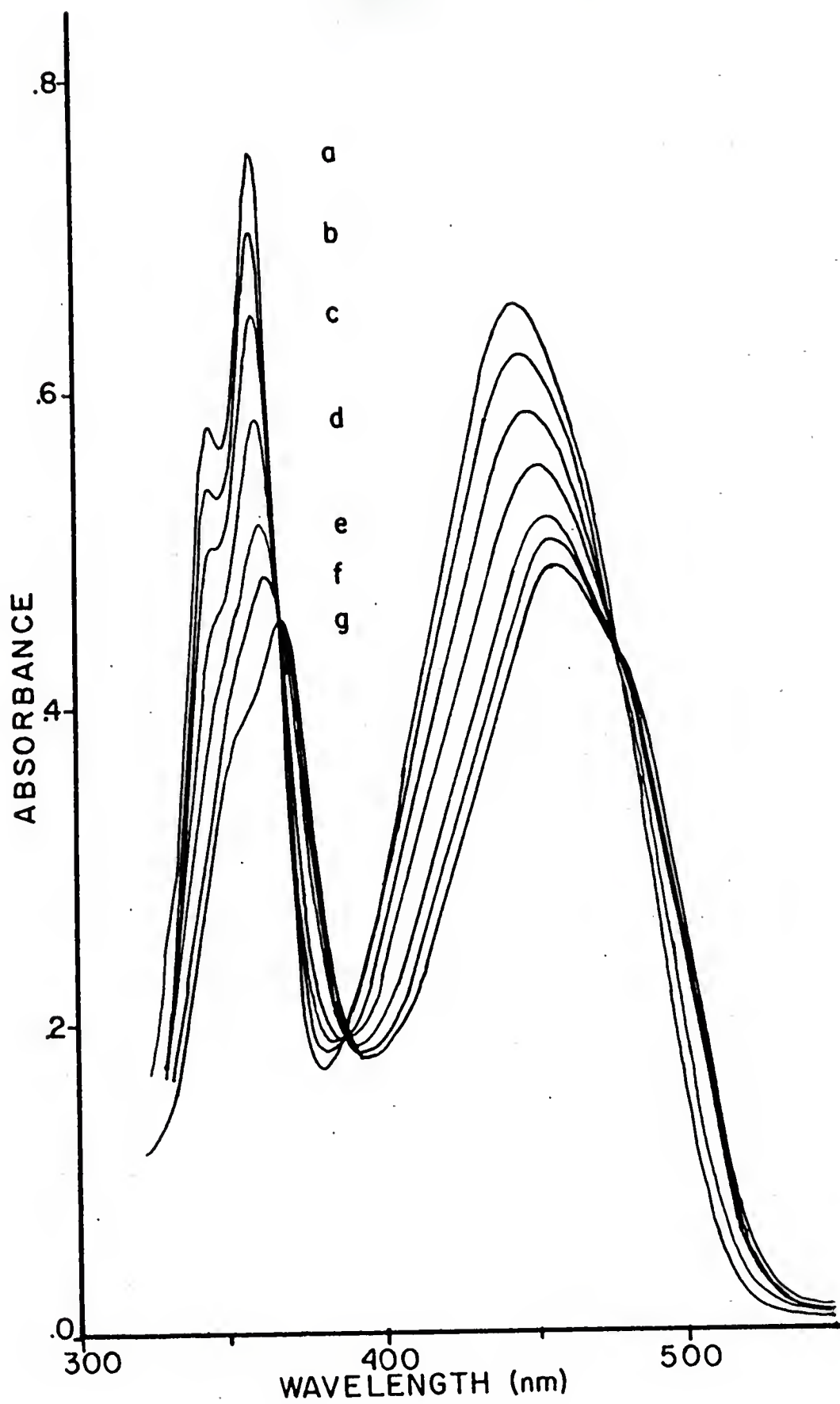


Figure 5. Absorptiometric titration of 3-aminoacridinium cation with DNA, magnesium salt, pH 5.90, 25.0°C. Background electrolyte:  $\text{Mg}(\text{O}_2\text{CCH}_3)_2$ , initial concentration, 0.010N

- a  $\text{P/D} = \text{--} ([\text{P}] = 0)$
- b  $\text{P/D} = 3.14$
- c  $\text{P/D} = 9.57$
- d  $\text{P/D} = 18.9$
- e  $\text{P/D} = 37.7$
- f  $\text{P/D} = 69.1$
- g  $\text{P/D} = 104$

P/D is the ratio of total DNA phosphate to total 3-aminoacridinium cation,  $\text{D} = 1.31 \times 10^{-7}$  moles.

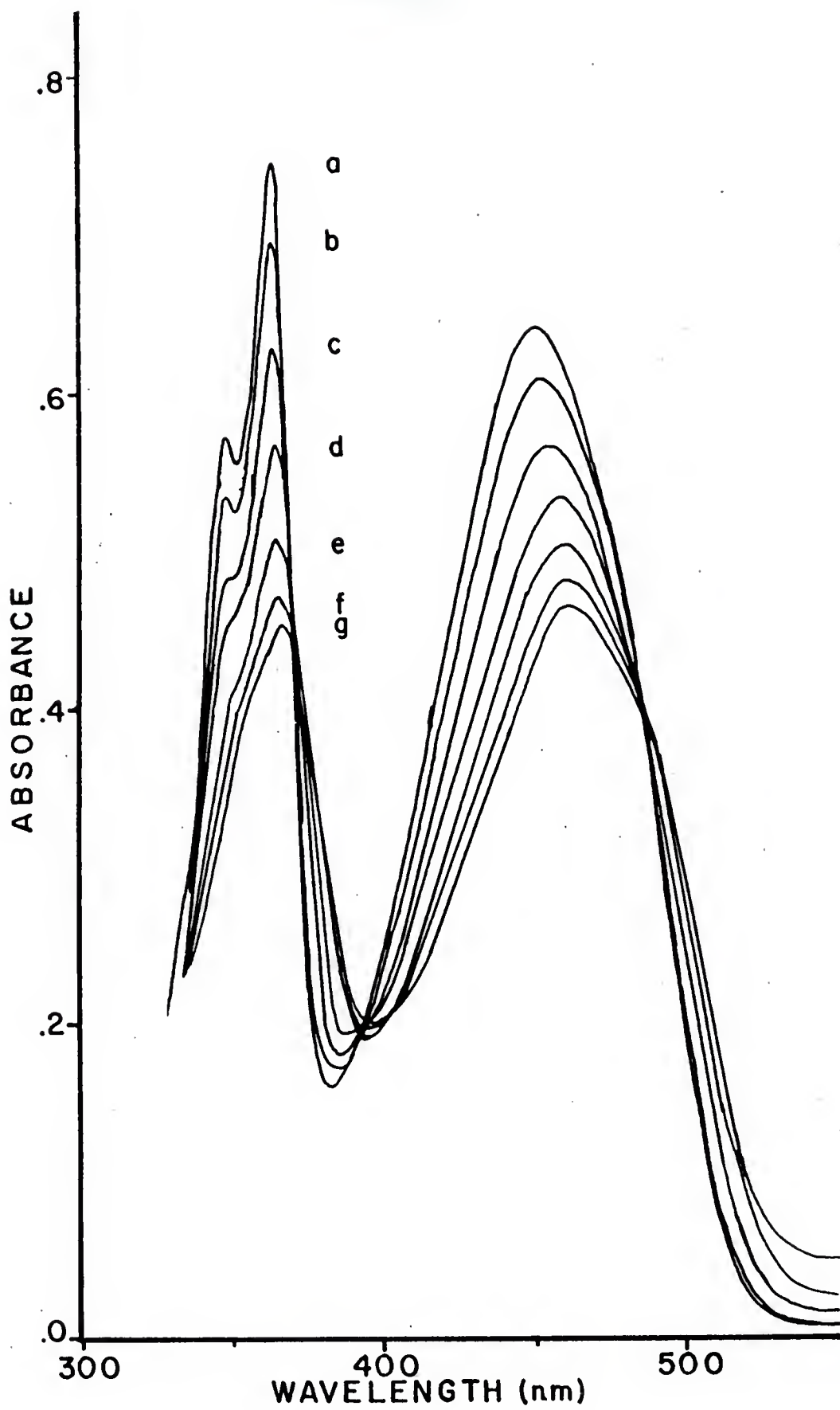


Figure 6. Absorptiometric titration of 3-aminoacridinium cation with DNA, magnesium salt, pH 5.90, 25.0°C. Background electrolyte:  $\text{Mg}(\text{O}_2\text{CCH}_3)_2$ , initial concentration, 0.00063N

- a  $\text{P/D} = -- ([\text{P}] = 0)$
- b  $\text{P/D} = 1.63$
- c  $\text{P/D} = 3.59$
- d  $\text{P/D} = 6.49$
- e  $\text{P/D} = 13.0$
- f  $\text{P/D} = 26.0$
- g  $\text{P/D} = 64.9$

P/D is the ratio of total DNA phosphate to total 3-aminoacridinium cation,  $\text{D} = 1.27 \times 10^{-7}$  moles.

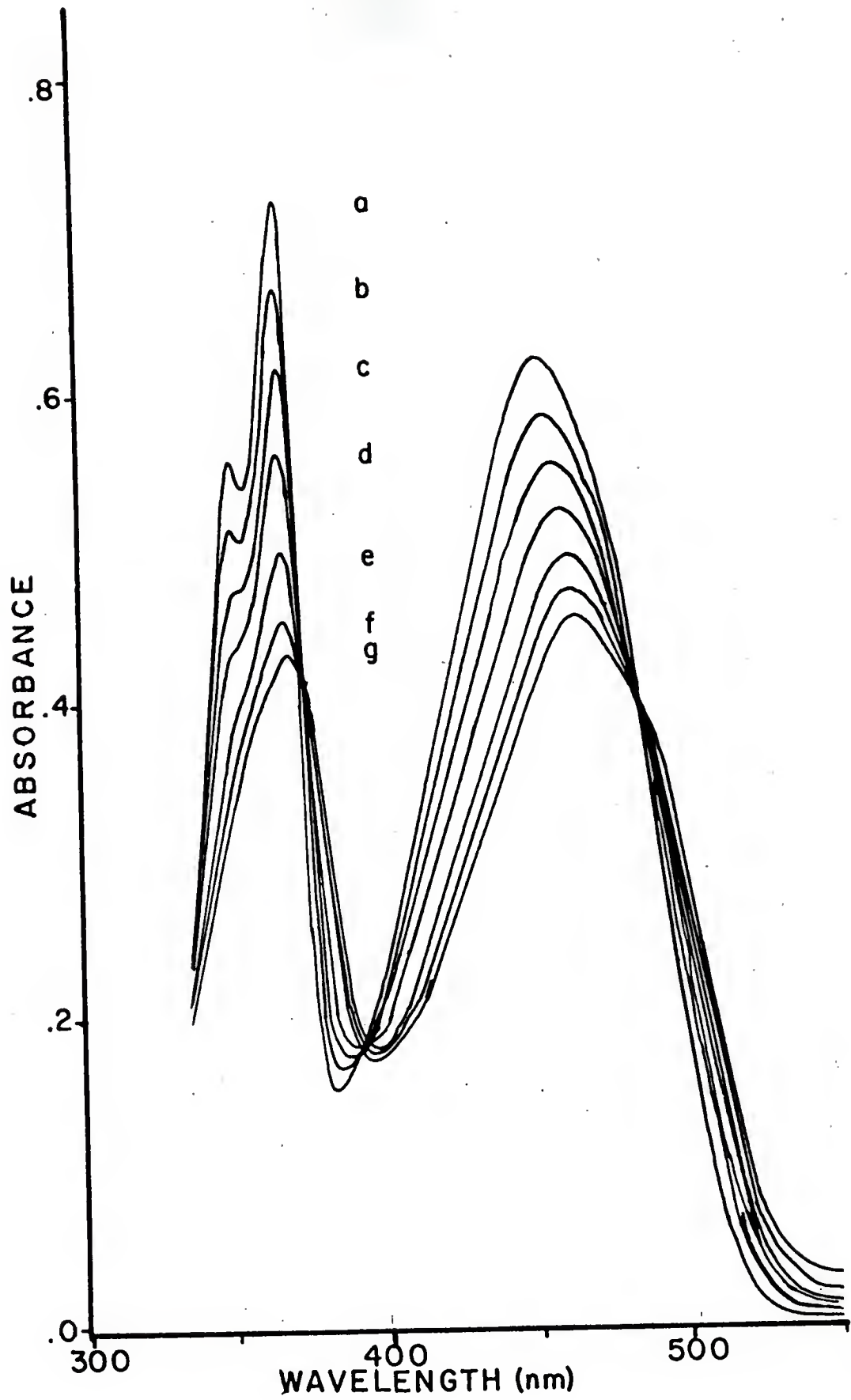




Figure 7.  $\log([BHP]/[BH])$  vs.  $\log([P_t/3]-[BHP])$  with 0.0025  $C_5H_2PO_4$  and  $6.3 \times 10^{-4}N$   $Mg(O_2CCH_3)_2$ , as supporting electrolytes, pH 5.90, 25.0°C

$\Delta$  0.0025M  $C_5H_2PO_4$

O  $6.3 \times 10^{-4}N$   $Mg(O_2CCH_3)_2$

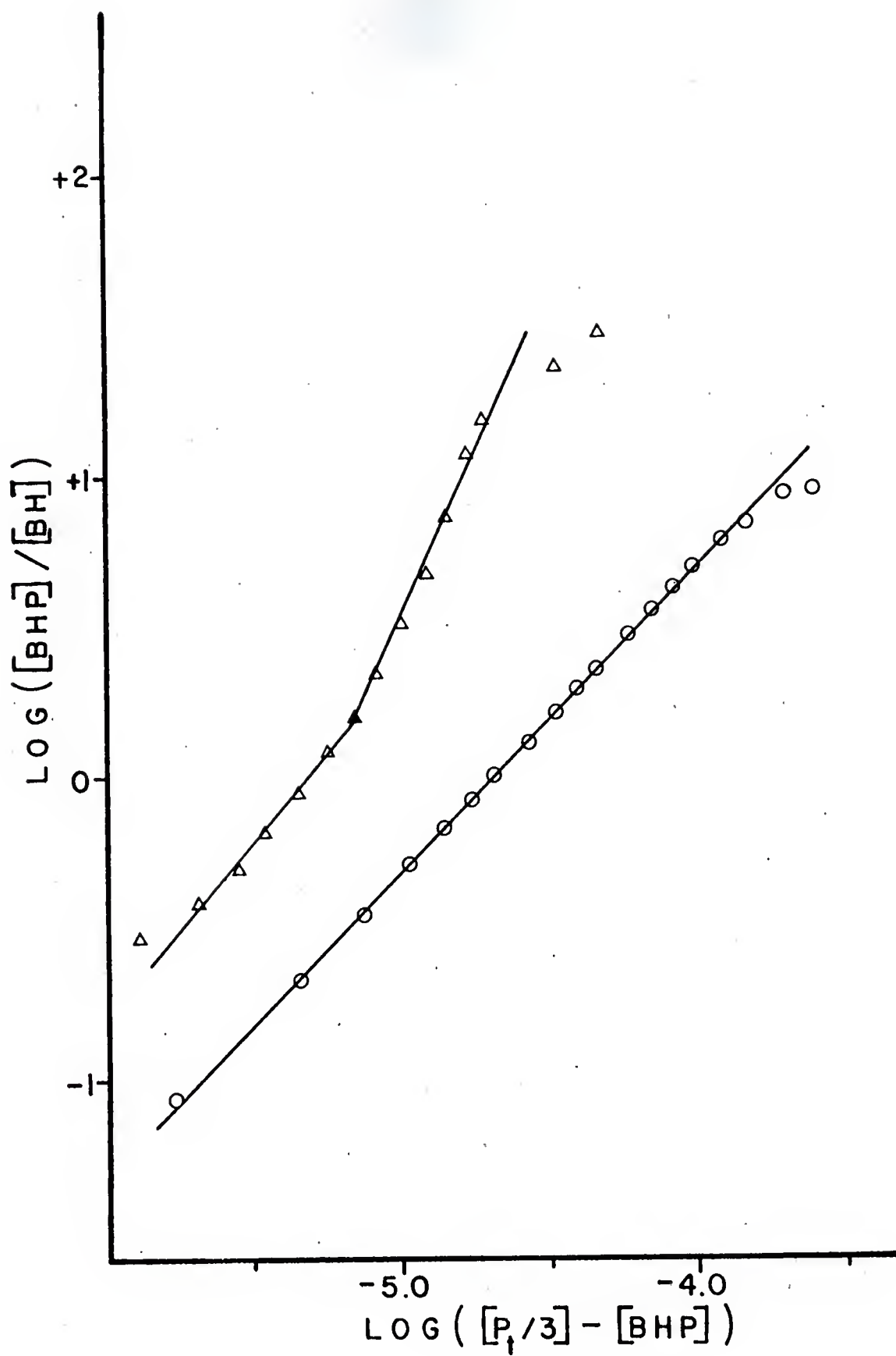


Figure 8.  $\text{Log}([\text{BHP}]/[\text{BH}])$  vs.  $\text{log}([\text{P}_t/3] - [\text{BHP}])$  with 0.15M  $\text{C}_5\text{H}_2\text{PO}_4$ , 0.010M  $\text{C}_5\text{H}_2\text{PO}_4$ , and 0.010N  $\text{Mg}(\text{O}_2\text{CCH}_3)_2$  as supporting electrolytes, pH 5.90, 25.0°C

- $\Delta$  0.15M  $\text{C}_5\text{H}_2\text{PO}_4$
- $\bullet$  0.01M  $\text{C}_5\text{H}_2\text{PO}_4$
- $\circ$  0.010N  $\text{Mg}(\text{O}_2\text{CCH}_3)_2$

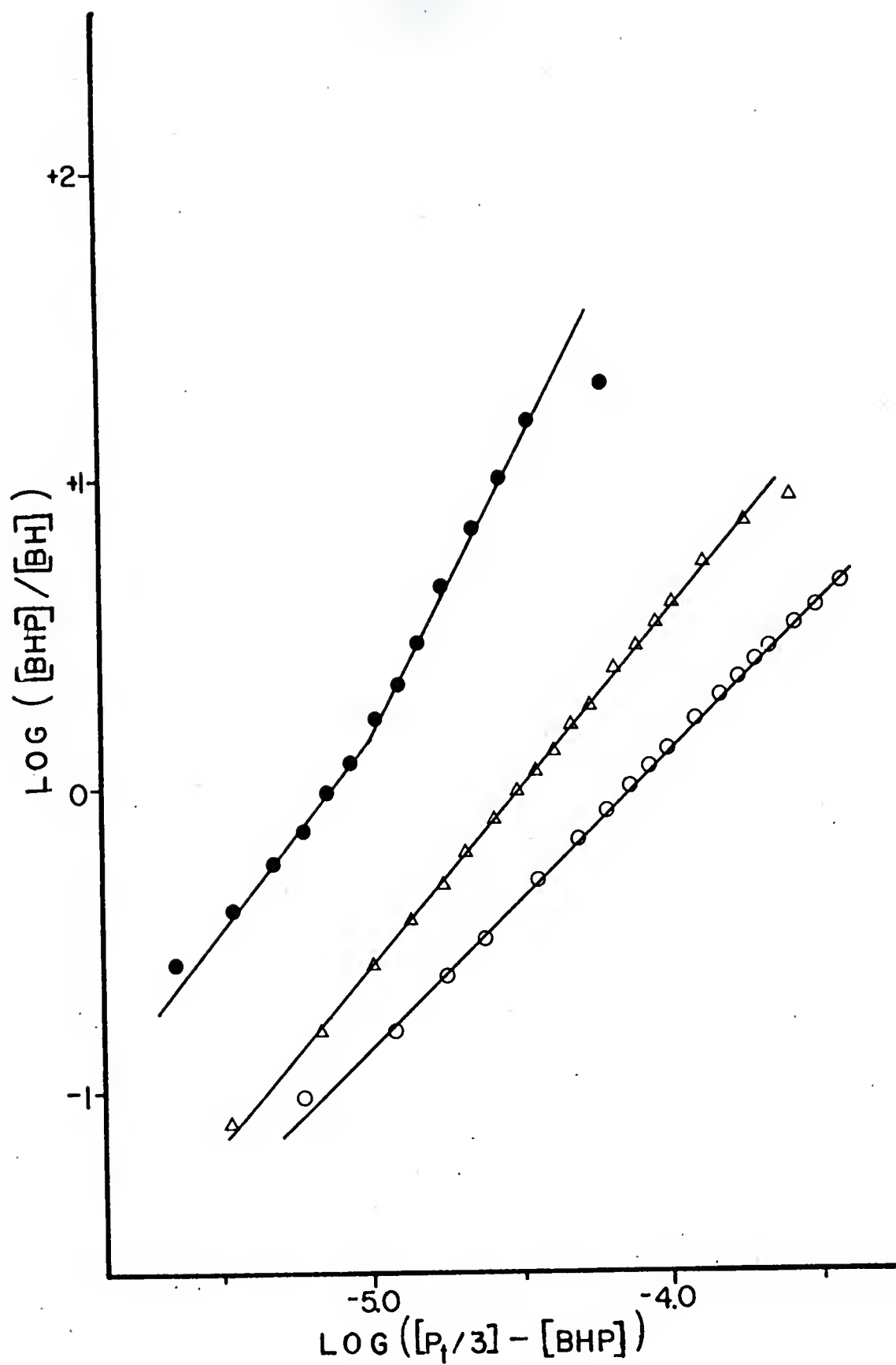


Figure 9.  $\text{Log}([\text{BHP}]/[\text{BH}])$  vs.  $\text{log}([\text{P}_t/2]-2[\text{BHP}])$  with 0.00  $\text{C}_5\text{H}_2\text{PO}_4$  and  $6.3 \times 10^{-4}\text{N}$   $\text{Mg}(\text{O}_2\text{CCH}_3)_2$  as supporting electrolytes, pH 5.90,  $25.0^\circ\text{C}$

$\Delta$  0.0025M  $\text{C}_5\text{H}_2\text{PO}_4$

O  $6.3 \times 10^{-4}\text{N}$   $\text{Mg}(\text{O}_2\text{CCH}_3)_2$

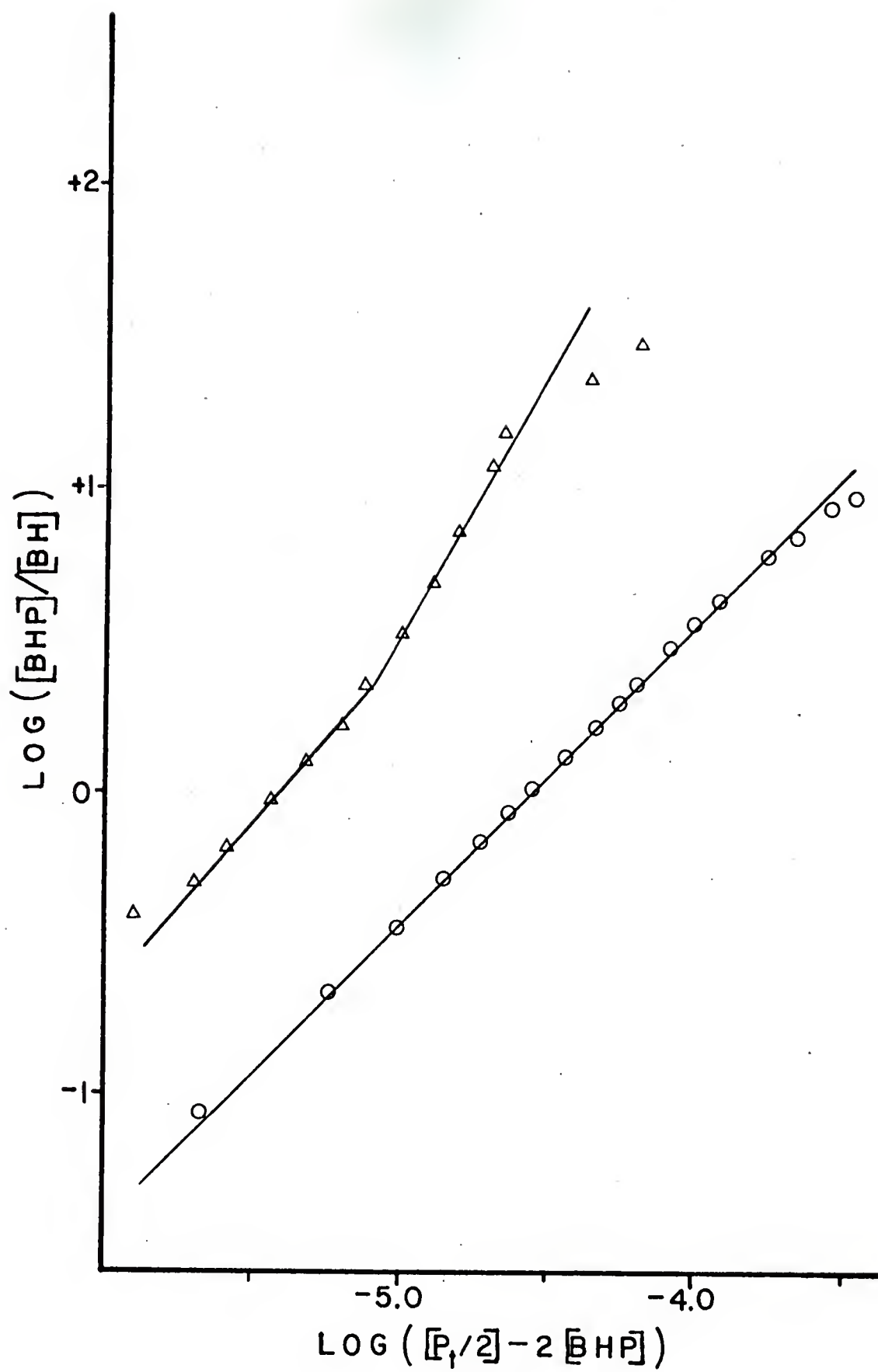


Figure 10.  $\text{Log}([\text{BHP}]/[\text{BH}])$  vs.  $\text{log}([\text{P}_t/2]-2[\text{BHP}])$  with 0.15  $\text{C}_5\text{H}_2\text{PO}_4$ , 0.01M  $\text{C}_5\text{H}_2\text{PO}_4$ , and 0.010N  $\text{Mg}(\text{O}_2\text{CCH}_3)_2$  as supporting electrolytes, pH 5.90, 25.0°C

$\Delta$  0.15M  $\text{C}_5\text{H}_2\text{PO}_4$

$\bullet$  0.010M  $\text{C}_5\text{H}_2\text{PO}_4$

$\circ$  0.010N  $\text{Mg}(\text{O}_2\text{CCH}_3)_2$



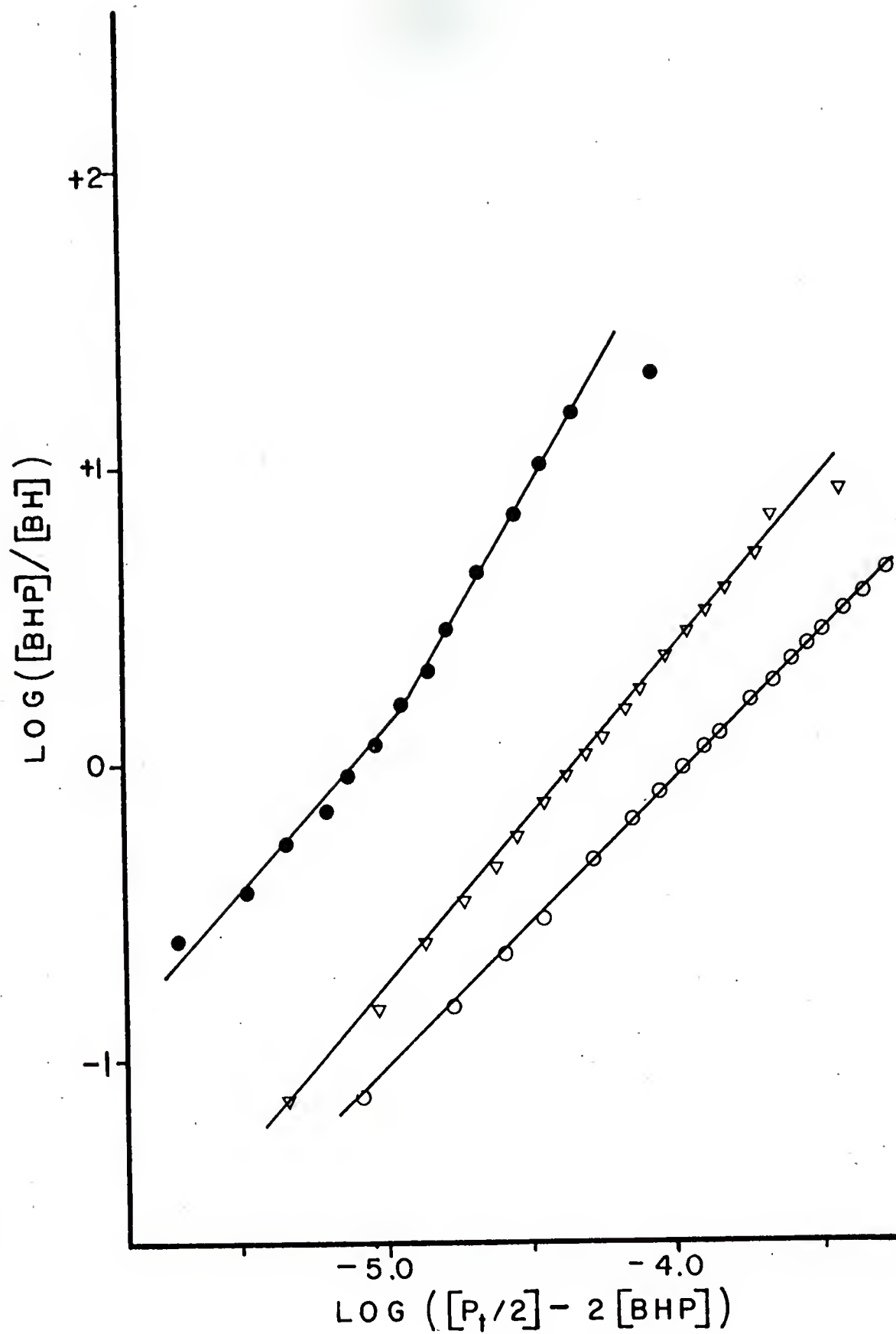


Figure 11. Log of the apparent association constant,  $K_s$ , for the surface binding of 3-aminoacridinium cation to DNA vs. the square root of the ionic strength,  $I^{1/2}$ .  $\text{NaH}_2\text{PO}_4$  and  $\text{LiH}_2\text{PO}_4$  as supporting electrolytes, pH 5.90, 25.0°C

X  $\text{NaH}_2\text{PO}_4$   
O  $\text{LiH}_2\text{PO}_4$

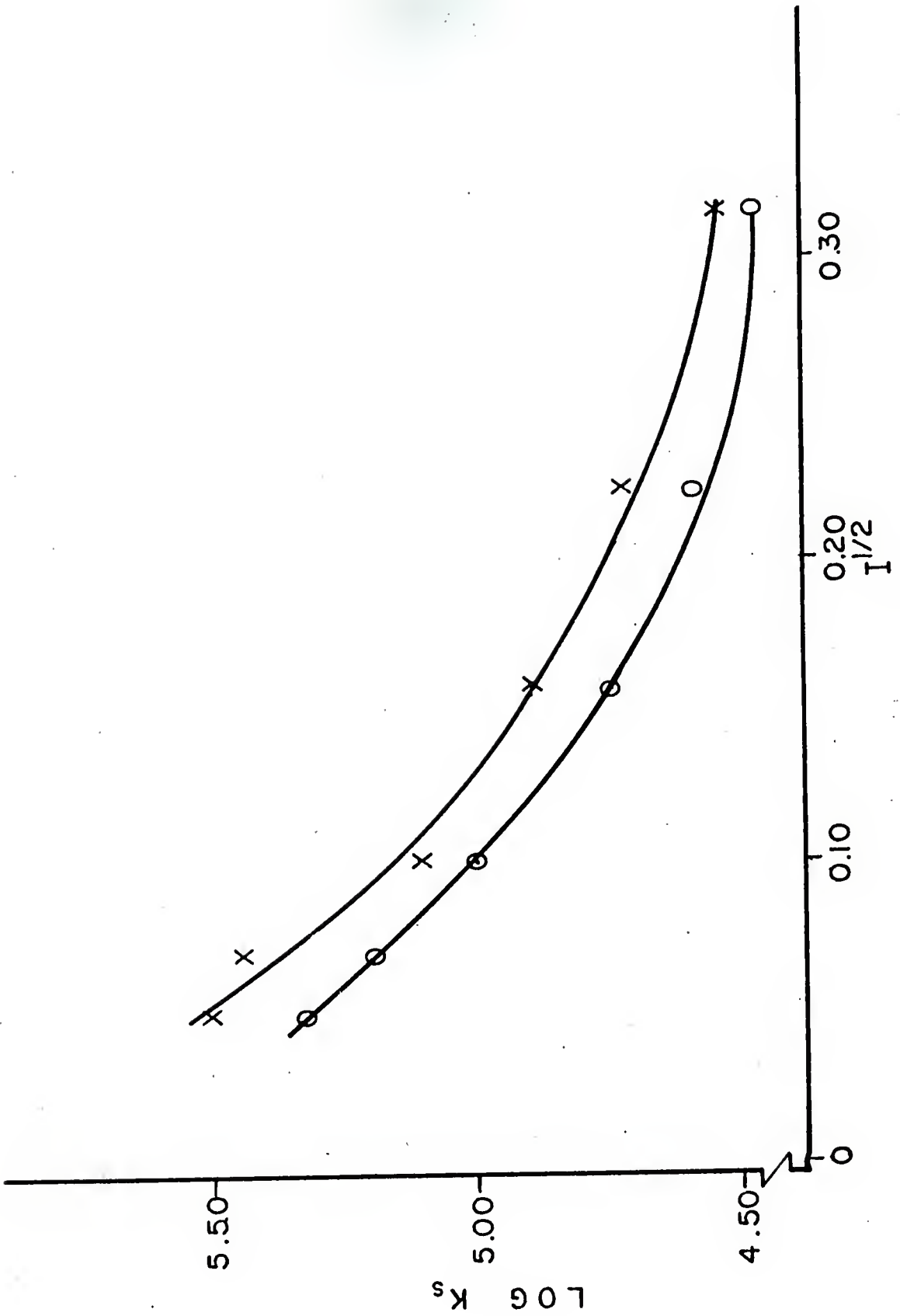


Figure 12. Log of the apparent association constant,  $K_s$ , for the surface binding of 3-aminoacridinium cation to DNA vs. the square root of the ionic strength,  $I^{1/2}$ .  $\text{KH}_2\text{PO}_4$  and  $\text{KO}_2\text{CCH}_3$  as supporting electrolytes, pH 5.90,  $25.0^\circ\text{C}$

X  $\text{KH}_2\text{PO}_4$   
O  $\text{KO}_2\text{CCH}_3$

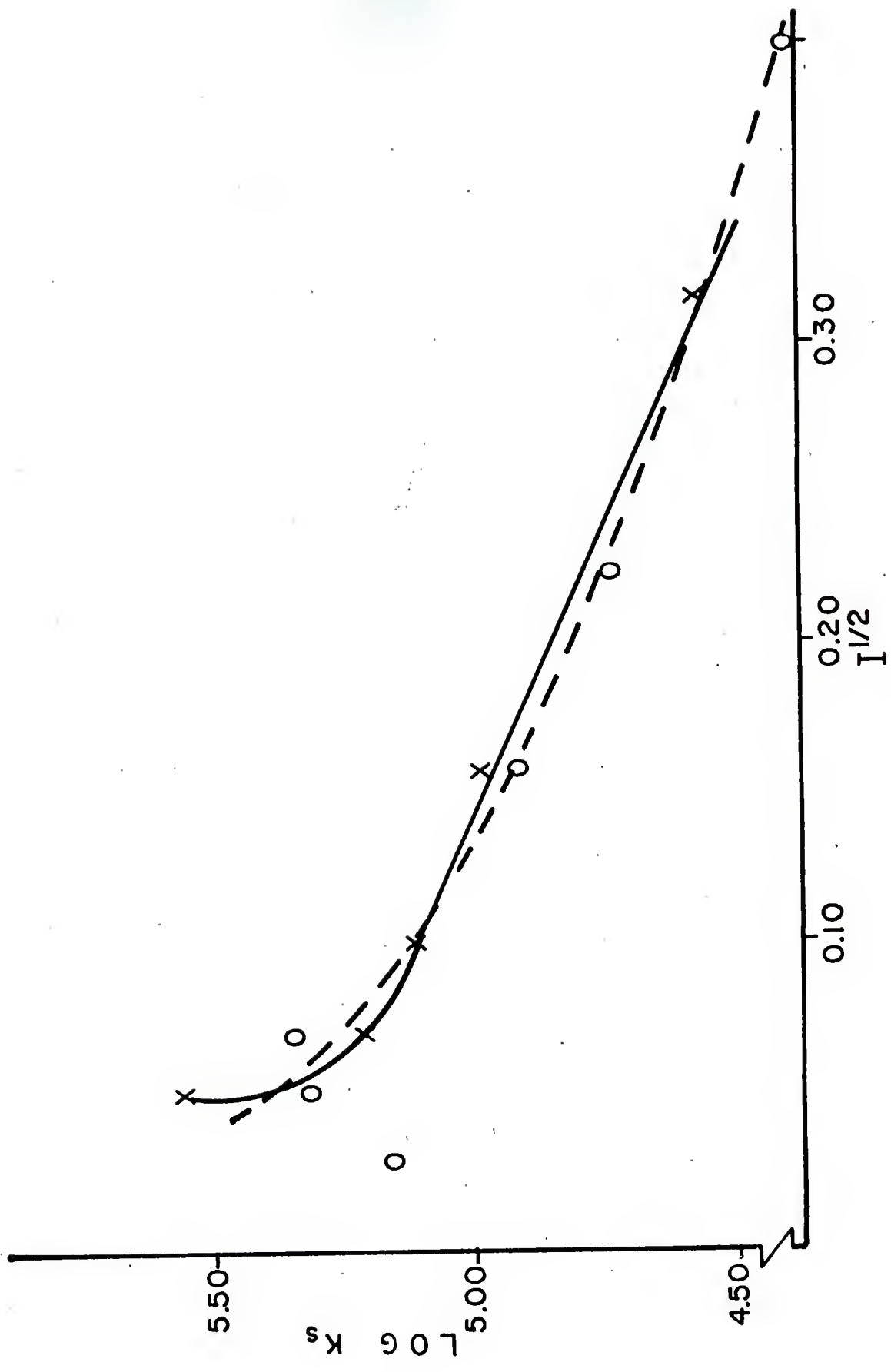


Figure 13. Log of the apparent association constant,  $K_s$ , for the surface binding of 3-aminoacridinium cation to DNA vs. the square root of the ionic strength,  $I^{1/2}$ .  $\text{KH}_2\text{PO}_4$  and  $\text{RbH}_2\text{PO}_4$  as supporting electrolytes, pH 5.90, 25.00°C

X  $\text{KH}_2\text{PO}_4$   
O  $\text{RbH}_2\text{PO}_4$

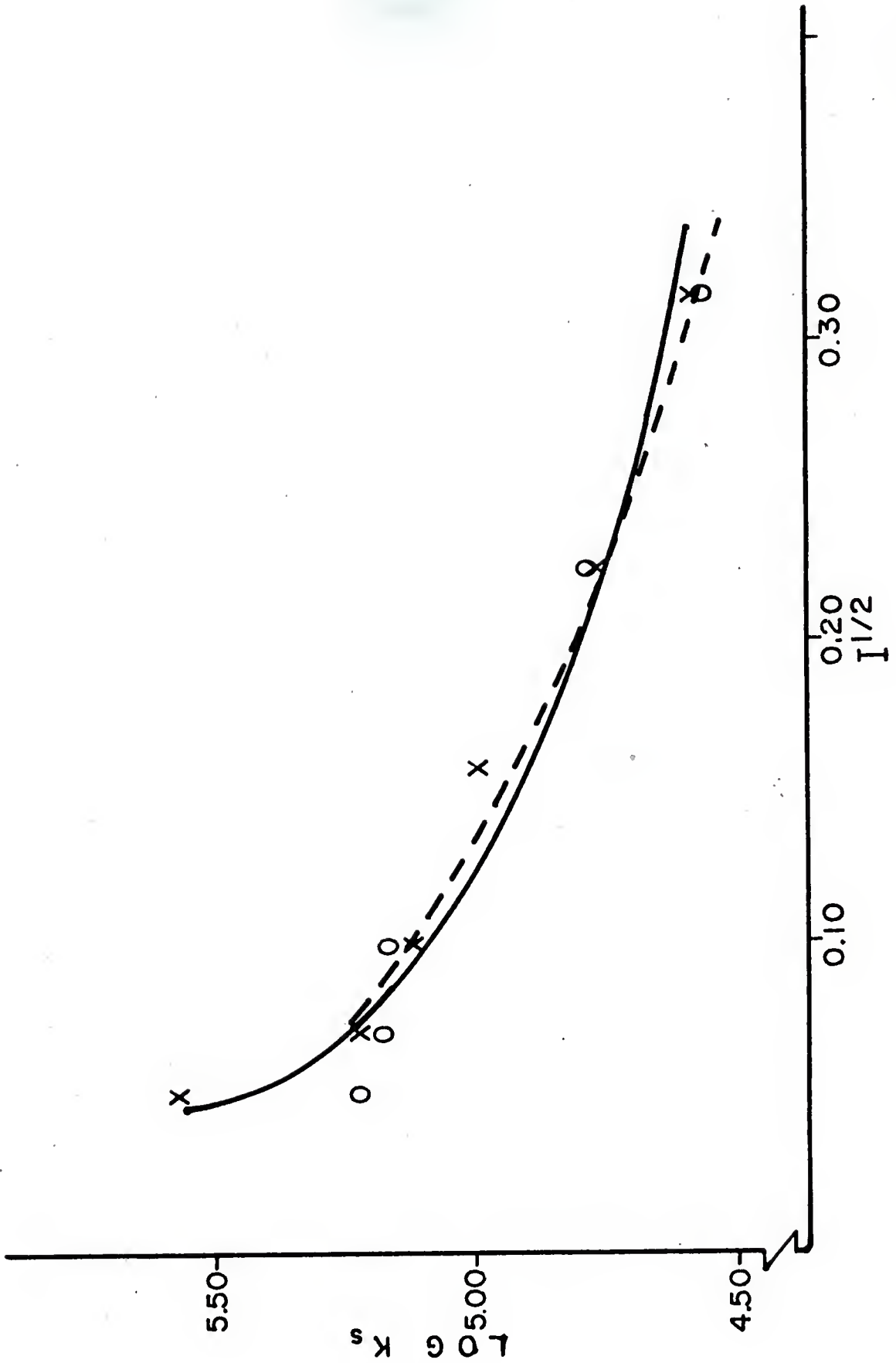




Figure 14. Log of the apparent association constant,  $K_s$ , for the surface binding of 3-aminoacridinium cation to DNA vs. the square root of the ionic strength,  $I^{1/2}$ .  $C_5H_2PO_4$  and  $(CH_3)_4NH_2PO_4$  as supporting electrolytes, pH 5.90, 25.0°C

X  $C_5H_2PO_4$   
O  $(CH_3)_4NH_2PO_4$

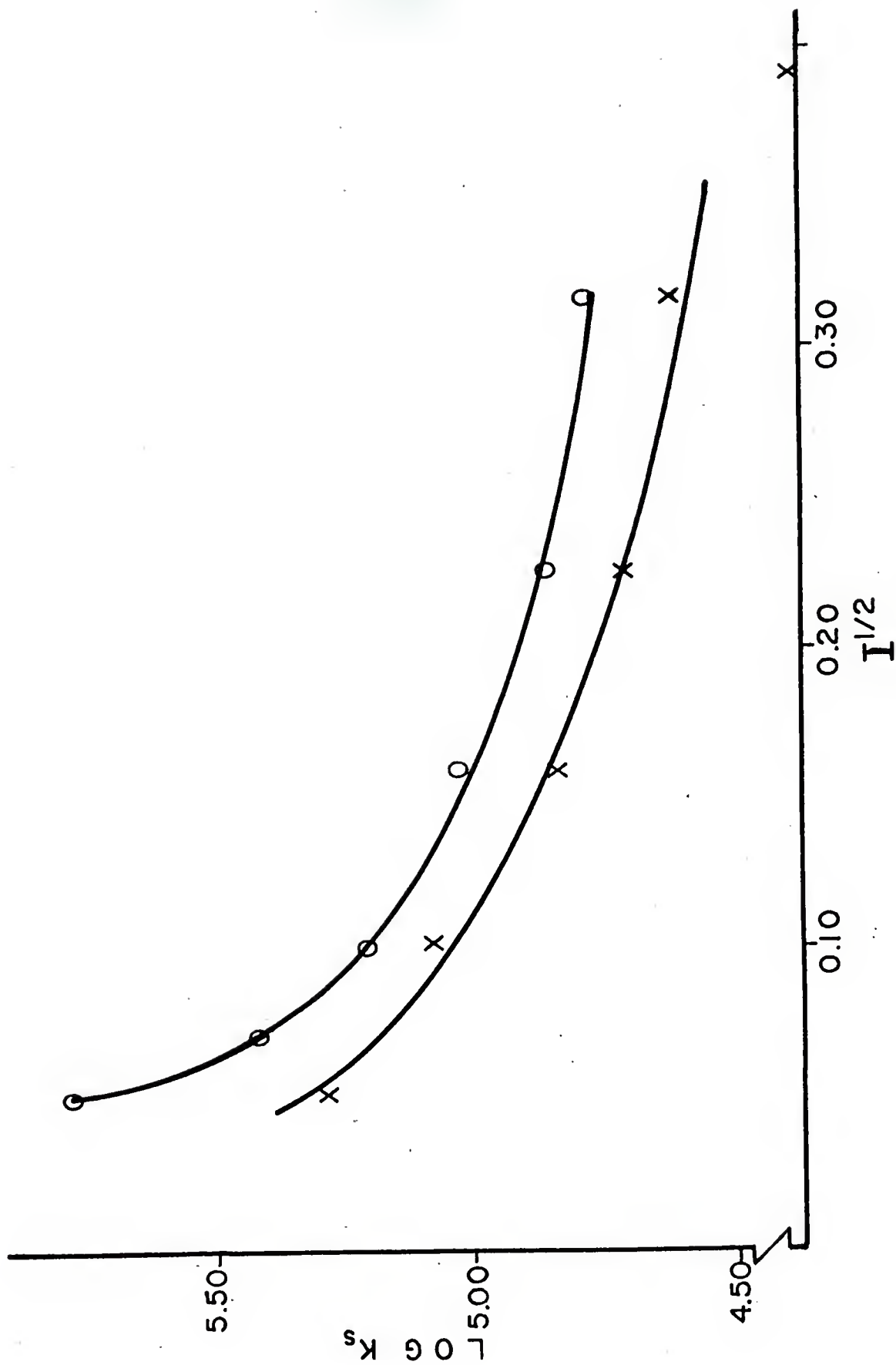


Figure 15. Log of the apparent association constant,  $K_s$ , for the surface binding of 3-aminoacridinium cation to DNA vs. the square root of the ionic strength,  $I^{1/2}$ .  $Mg(O_2CCH_3)_2$  and  $Ca(O_2CCH_3)_2$  as supporting electrolytes, pH 5.90, 25.0°C

X  $Mg(O_2CCH_3)_2$   
 O  $Ca(O_2CCH_3)_2$

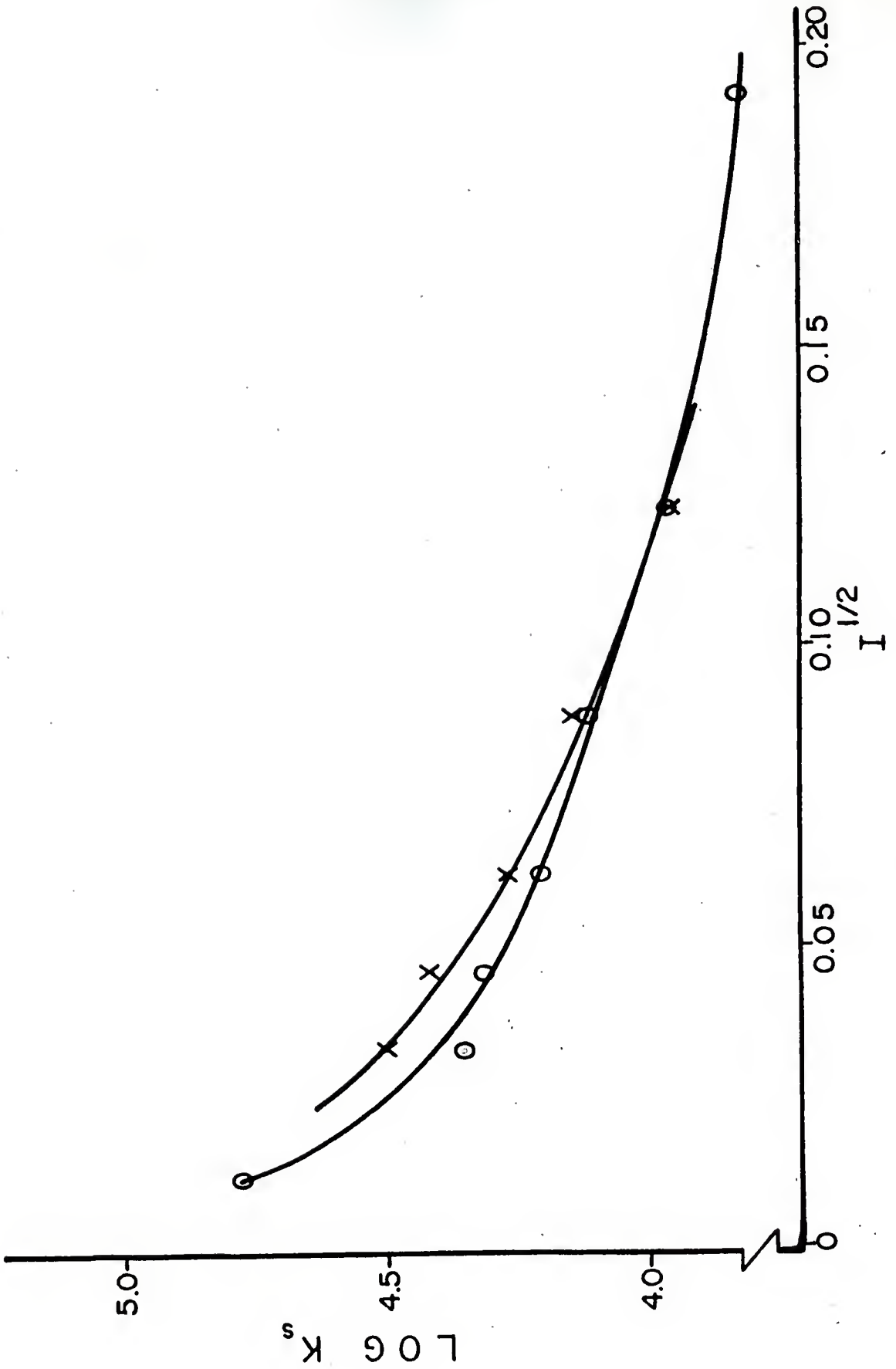


Figure 16. Log of the apparent association constant,  $K_s$ , for the surface binding of 3-aminoacridinium cation to DNA vs. the square root of the ionic strength,  $I^{1/2}$ .  $\text{Sr}(\text{O}_2\text{CCH}_3)_2$  and  $\text{Ba}(\text{O}_2\text{CCH}_3)_2$  as supporting electrolytes, pH 5.90, 25.0°C

X  $\text{Sr}(\text{O}_2\text{CCH}_3)_2$   
 O  $\text{Ba}(\text{O}_2\text{CCH}_3)_2$

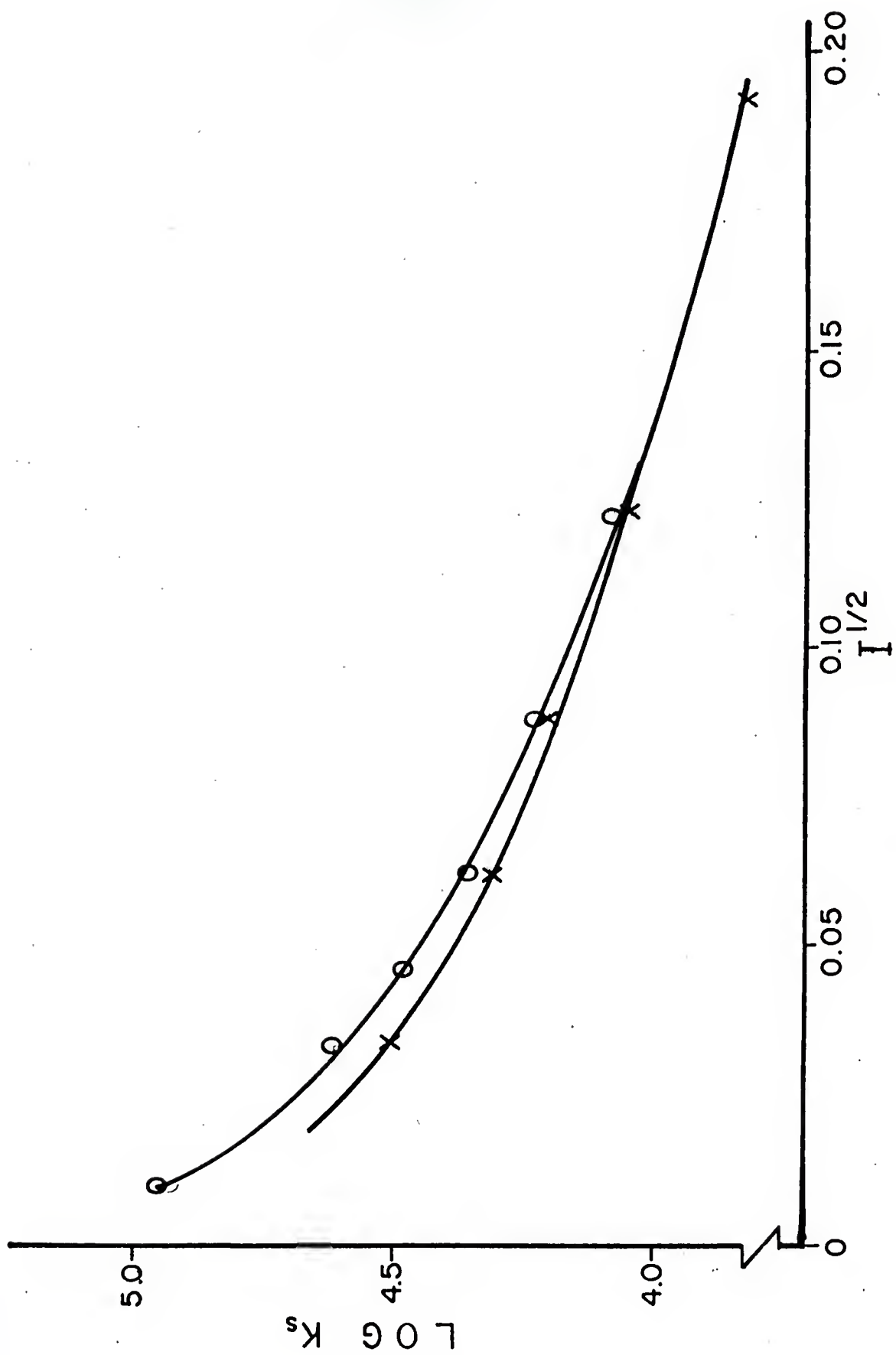


Figure 17. Log of the apparent association constant,  $K_I$ , for the intercalative binding of 3-aminoacridinium cation to DNA vs. the square root of the ionic strength,  $I^{1/2}$ .  $\text{LiH}_2\text{PO}_4$  and  $\text{NaH}_2\text{PO}_4$  as supporting electrolytes, pH 5.90, 25.0°C

O  $\text{LiH}_2\text{PO}_4$

X  $\text{NaH}_2\text{PO}_4$



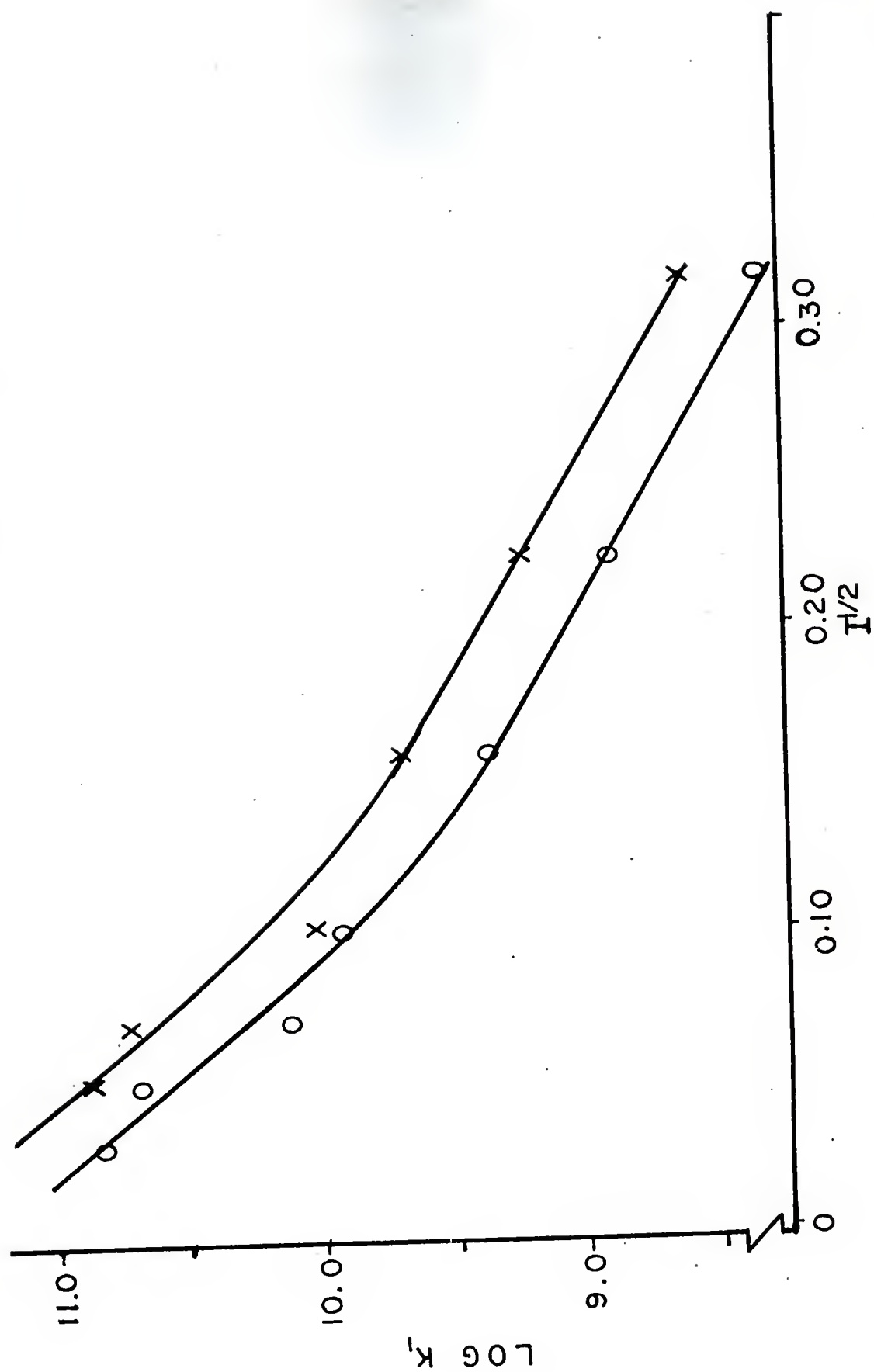


Figure 18. Log of the apparent association constant,  $K_I$ , for the intercalative binding of 3-aminoacridinium cation to DNA vs. the square root of the ionic strength,  $I^{1/2}$ .  $KH_2PO_4$  and  $KO_2CCH_3$  as supporting electrolytes, pH 5.90, 25.0°C

X  $KH_2PO_4$   
O  $KO_2CCH_3$

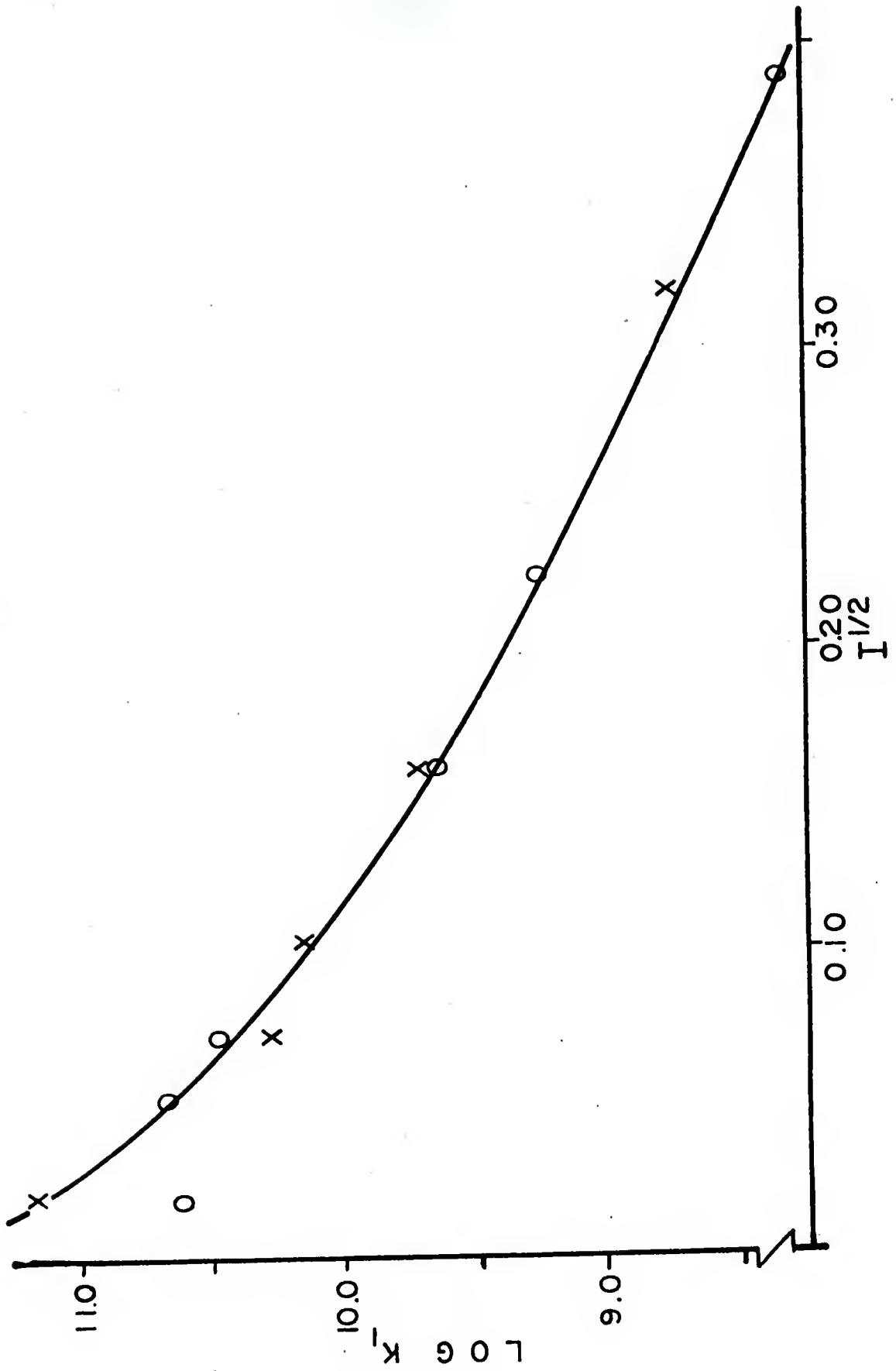


Figure 19. Log of the apparent association constant,  $K_I$ , for the intercalative binding of 3-aminoacridinium cation to DNA vs. the square root of the ionic strength,  $I^{1/2}$ .  $KH_2PO_4$  and  $RbH_2PO_4$  as supporting electrolytes, pH 5.90, 25.0°C

X  $KH_2PO_4$

O  $RbH_2PO_4$

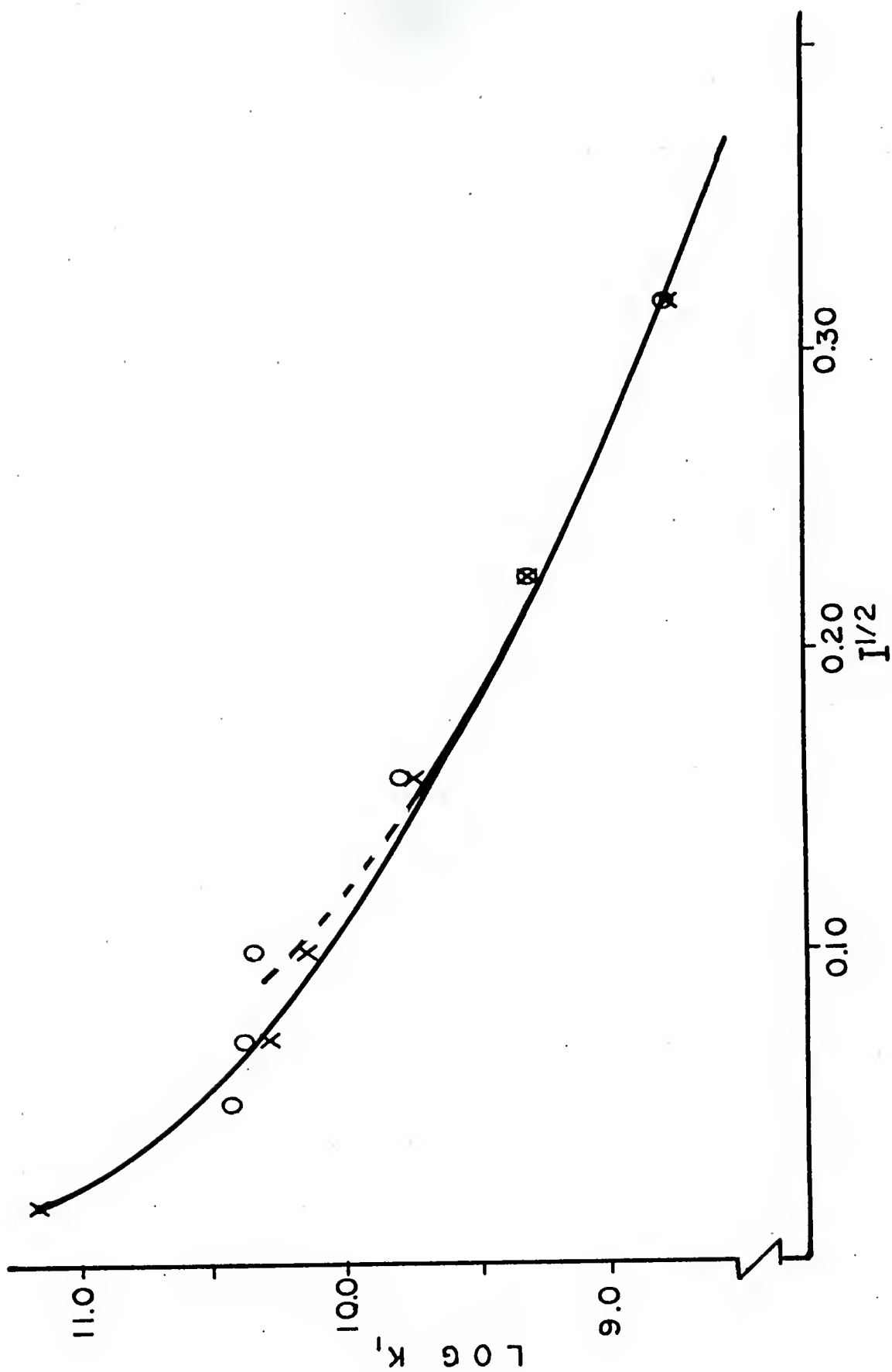


Figure 20. Log of the apparent association constant,  $K_I$ , for the intercalative binding of 3-aminoacridinium cation to DNA vs. the square root of the ionic strength,  $I^{1/2}$ .  $\text{CsH}_2\text{PO}_4$  and  $(\text{CH}_3)_4\text{NH}_2\text{PO}_4$  as supporting electrolytes, pH 5.90, 25.0°C

X  $\text{CsH}_2\text{PO}_4$   
O  $(\text{CH}_3)_4\text{NH}_2\text{PO}_4$

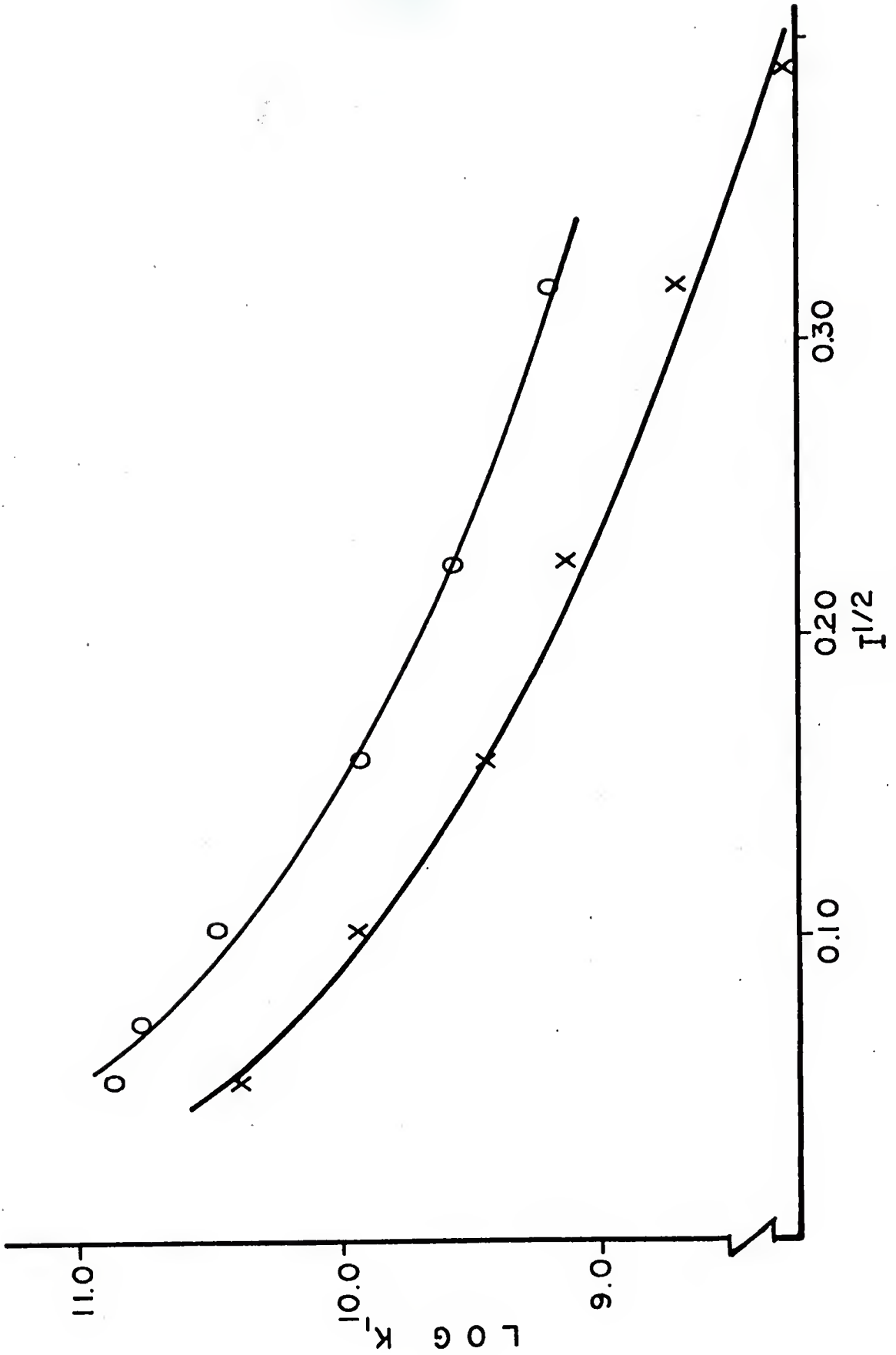




Figure 21. Log of the corrected association constant,  $K_s'$ , for the surface binding of 3-aminoacridinium cation to DNA vs. the square root of the activity of solution,  $I^{1/2}$ .  $\text{LiH}_2\text{PO}_4$ ,  $\text{NaH}_2\text{PO}_4$ , and  $\text{KH}_2\text{PO}_4$  as supporting electrolytes, pH 5.90, 25.0°C

○  $\text{LiH}_2\text{PO}_4$   
●  $\text{NaH}_2\text{PO}_4$   
□  $\text{KH}_2\text{PO}_4$

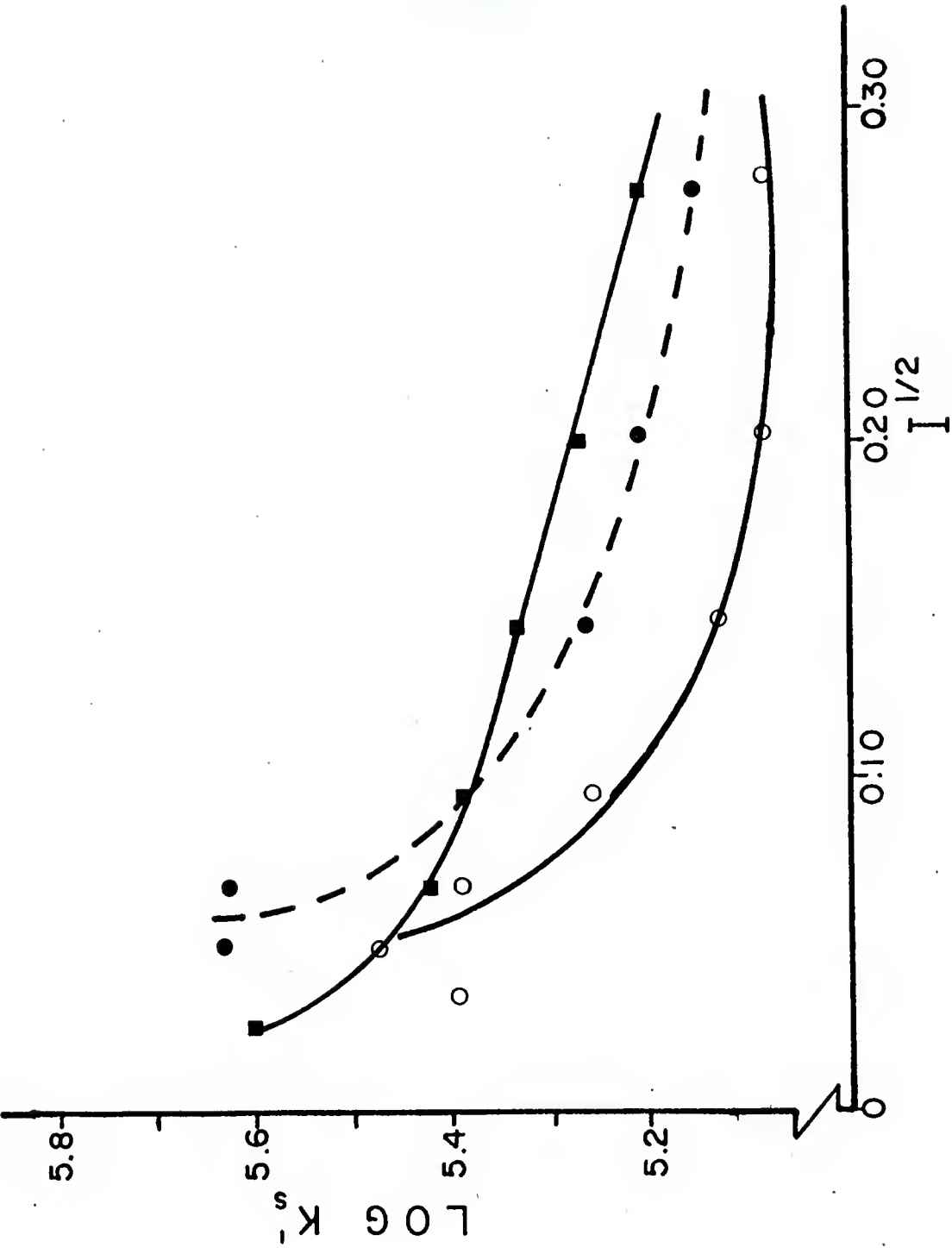


Figure 22. Log of the corrected association constant,  $K_s'$ , for the surface binding of 3-aminoacridinium cation to DNA vs. the square root of the activity of solution,  $I^{1/2}$ .  $RbH_2PO_4$ ,  $CsH_2PO_4$ , and  $(CH_3)_4NH_2PO_4$  as supporting electrolytes, pH 5.90, 25.0°C

○  $RbH_2PO_4$   
 ●  $CsH_2PO_4$   
 ■  $(CH_3)_4NH_2PO_4$

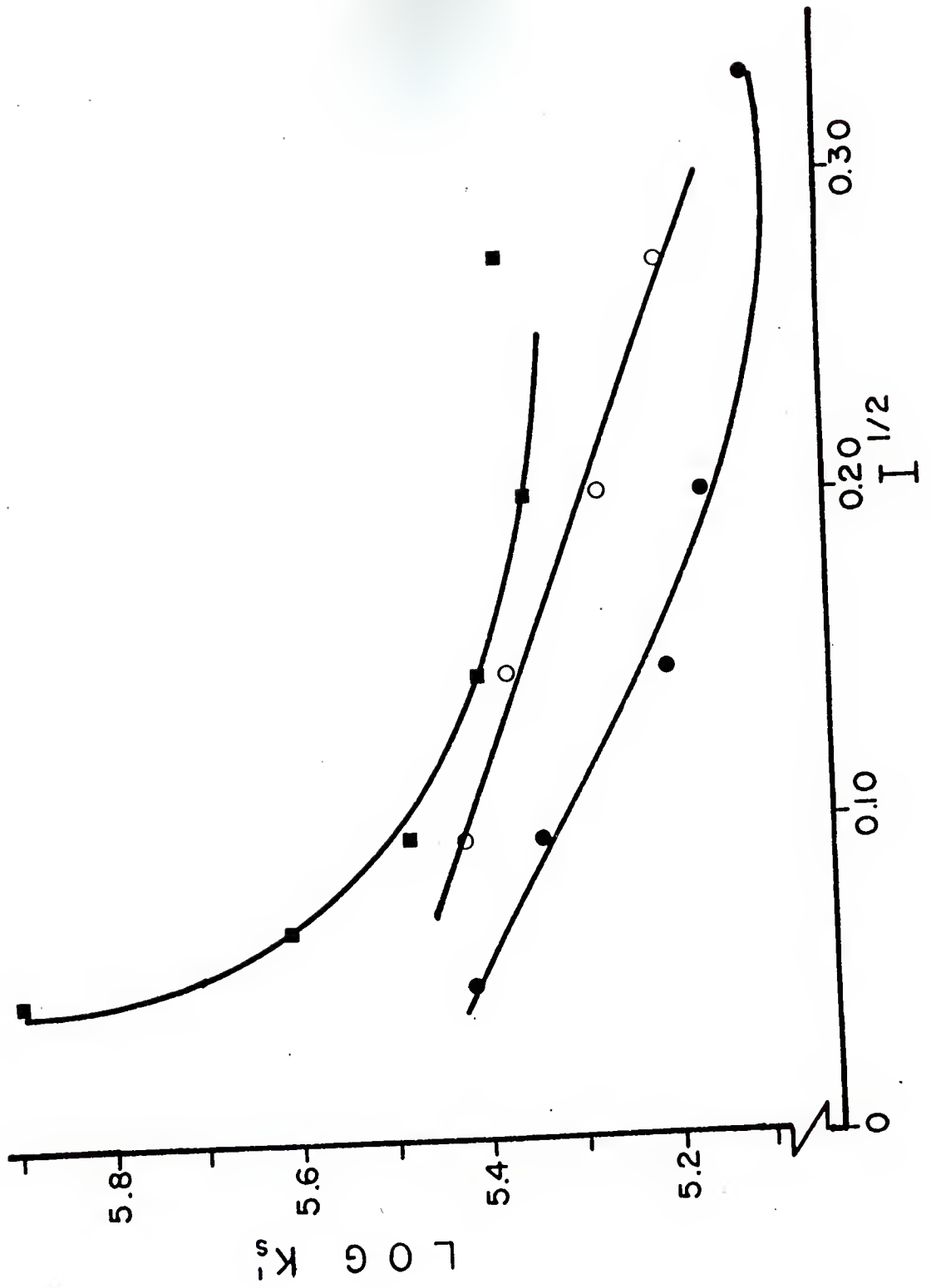


Figure 23. Log of the corrected association constant,  $K_s'$ , for the surface binding of 3-aminoacridinium cation to DNA vs. the square root of the activity of solution,  $I^{1/2}$ .  $Mg(O_2CCH_3)_2$  and  $Ca(O_2CCH_3)_2$  as supporting electrolytes, pH 5.90, 25.0°C

○  $Mg(O_2CCH_3)_2$

●  $Ca(O_2CCH_3)_2$

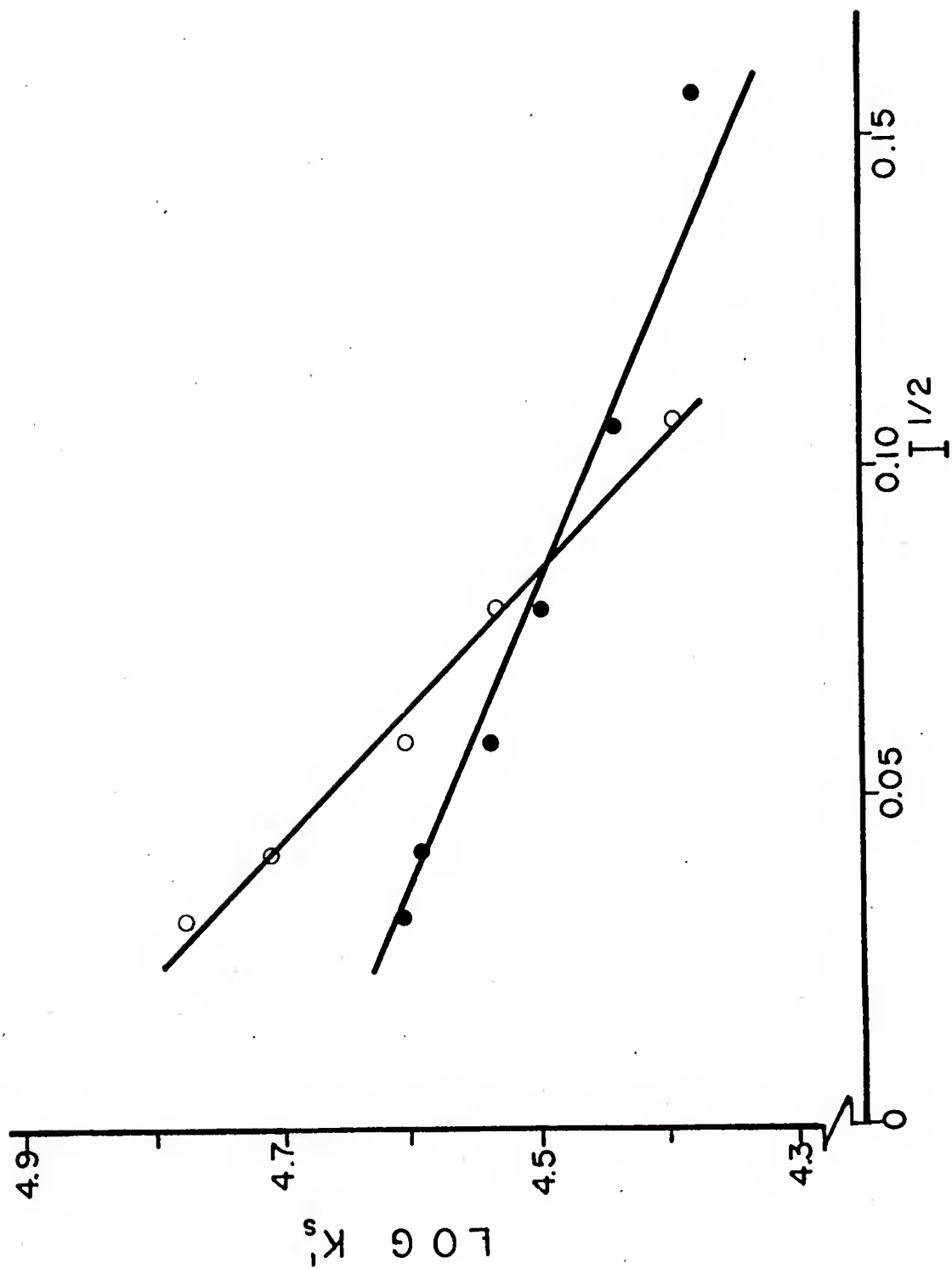


Figure 24. Log of the corrected association constant,  $K_s'$ , for the surface binding of 3-aminoacridinium cation to DNA vs. the square root of the activity of solution,  $I^{1/2}$ .  $Sr(O_2CCH_3)_2$  and  $Ba(O_2CCH_3)_2$  as supporting electrolytes, pH 5.90, 25.0°C

○  $Sr(O_2CCH_3)_2$

●  $Ba(O_2CCH_3)_2$



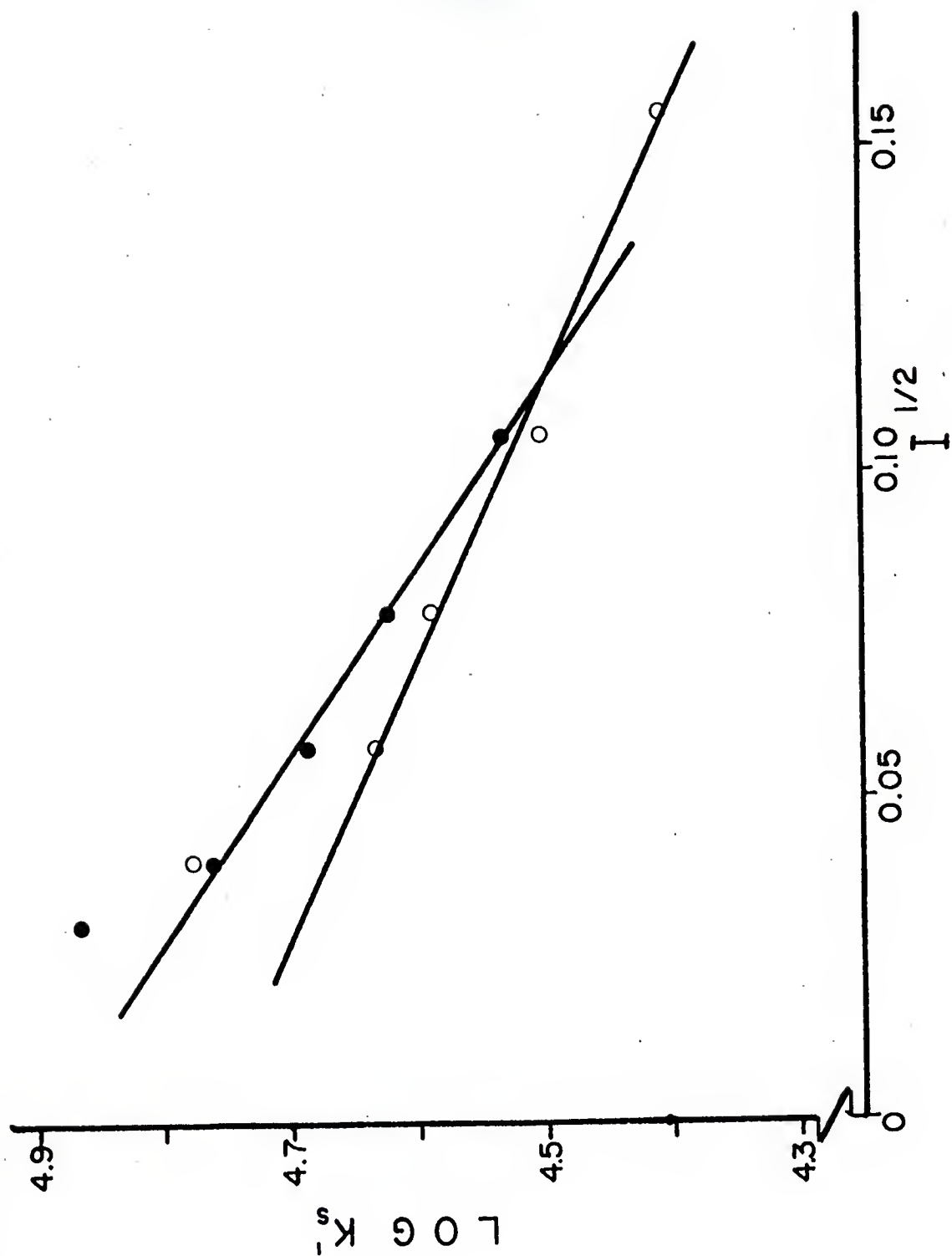


Figure 25. Absorptiometric titration of 7-aminoquinolinium cation with DNA, potassium salt, pH 5.90, 25.0°C. Background electrolyte:  $\text{KH}_2\text{PO}_4$ , initial concentration, 0.0050M

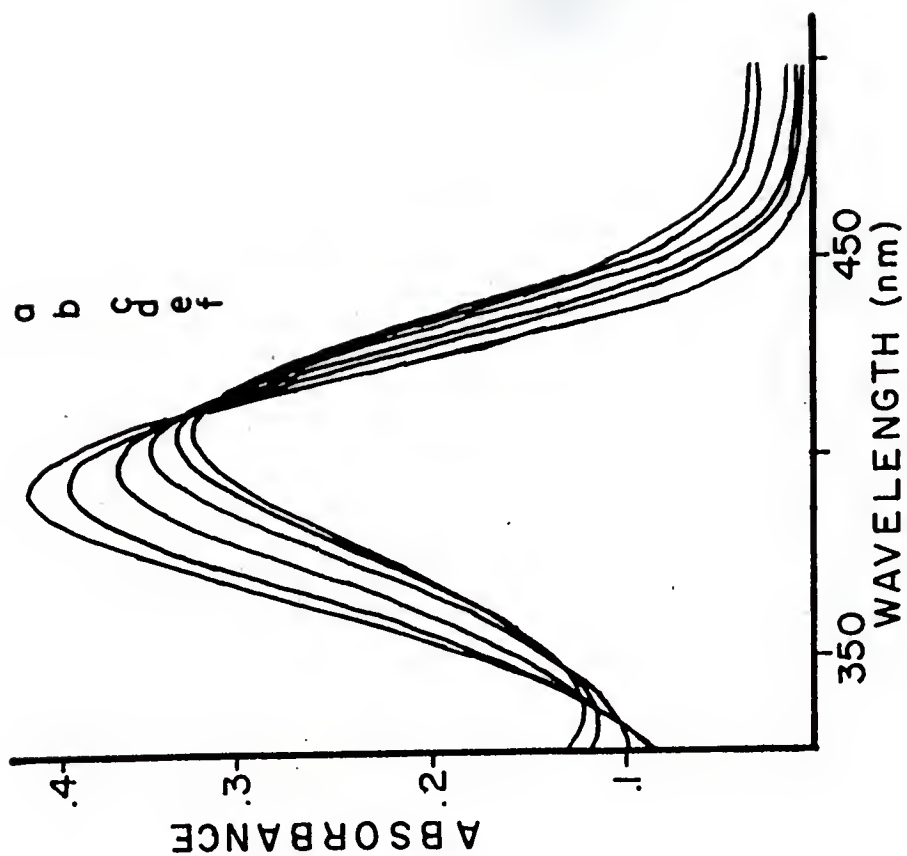
A Absorbances of individual spectra normalized to 0.00 at 500 nm

B Individual spectra as recorded

|   |                                           |
|---|-------------------------------------------|
| a | $[\text{P}/\text{D}] = -([\text{P}] = 0)$ |
| b | $[\text{P}/\text{D}] = 7.56$              |
| c | $[\text{P}/\text{D}] = 18.9$              |
| d | $[\text{P}/\text{D}] = 37.8$              |
| e | $[\text{P}/\text{D}] = 75.6$              |
| f | $[\text{P}/\text{D}] = 105.8$             |

P/D is the ratio of total DNA phosphate to total 7-aminoquinolinium cation,  $\text{D} = 1.08 \times 10^{-7}$  moles.

B



A

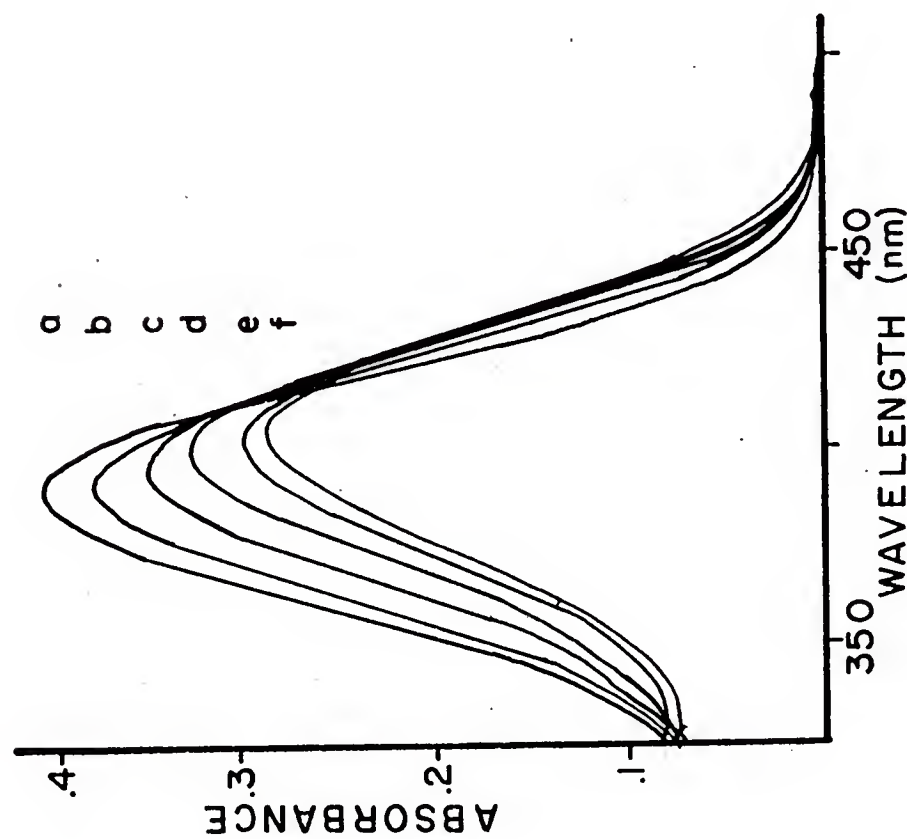


Figure 26.  $\log([AHP]/[AH])$  vs.  $\log([P_t/3]-[AHP])$  for the binding of 7-amino-quinolinium cation to DNA at 15.0°C, 25.0°C, and 35.0°C, pH 5.90, 0.010M  $KH_2PO_4$  as supporting electrolyte

15.0°C

25.0°C

35.0°C

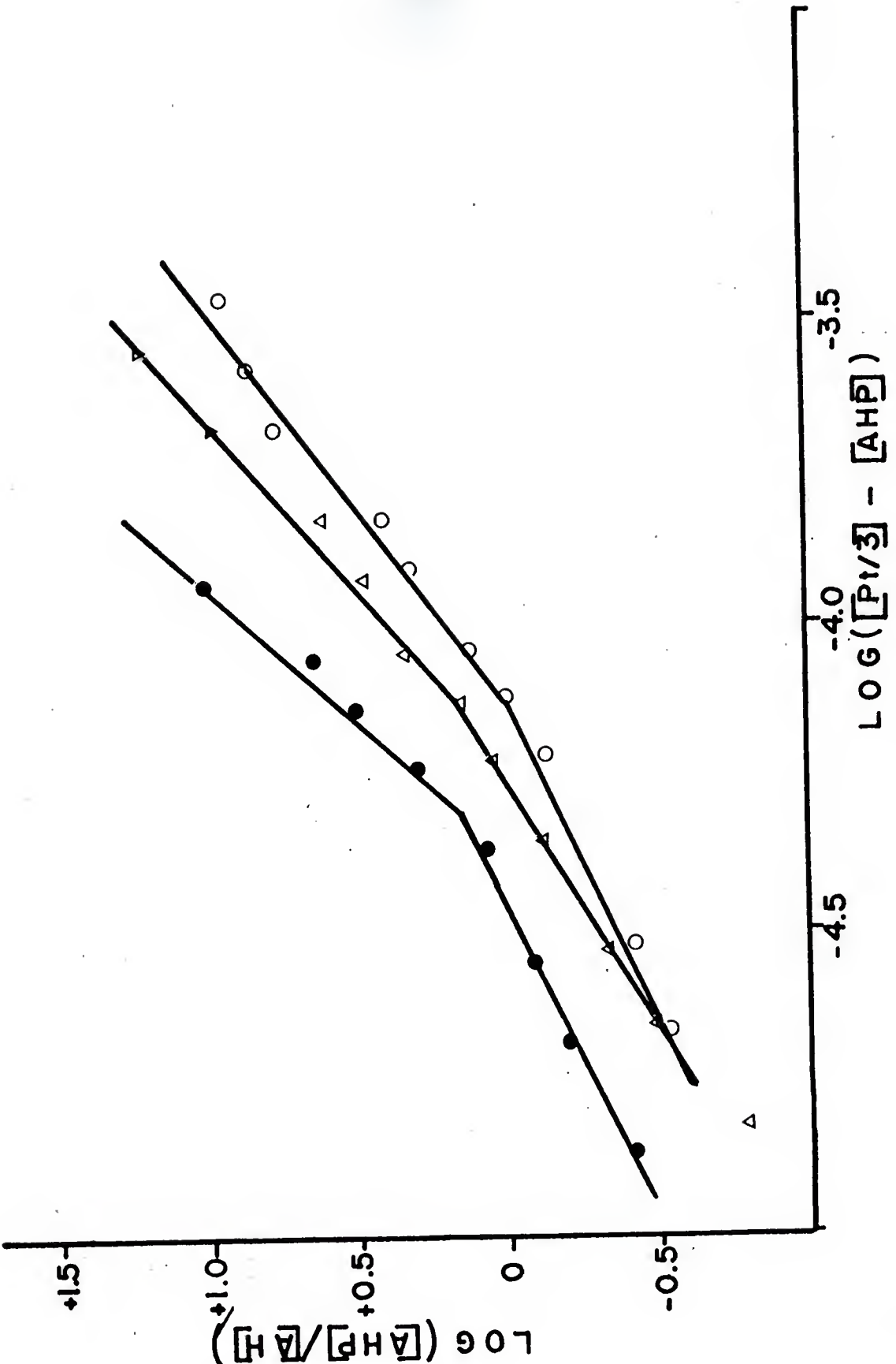


Figure 27.  $\log([AHP]/[AH])$  vs.  $\log([Pt/2]-2[AHP])$  for the binding of 7-amino-quinolinium cation to DNA at 15.0°C, 25.0°C, and 35.0°C, pH 5.90, 0.010M  $KH_2PO_4$  as supporting electrolyte.

15.0°C

25.0°C

35.0°C

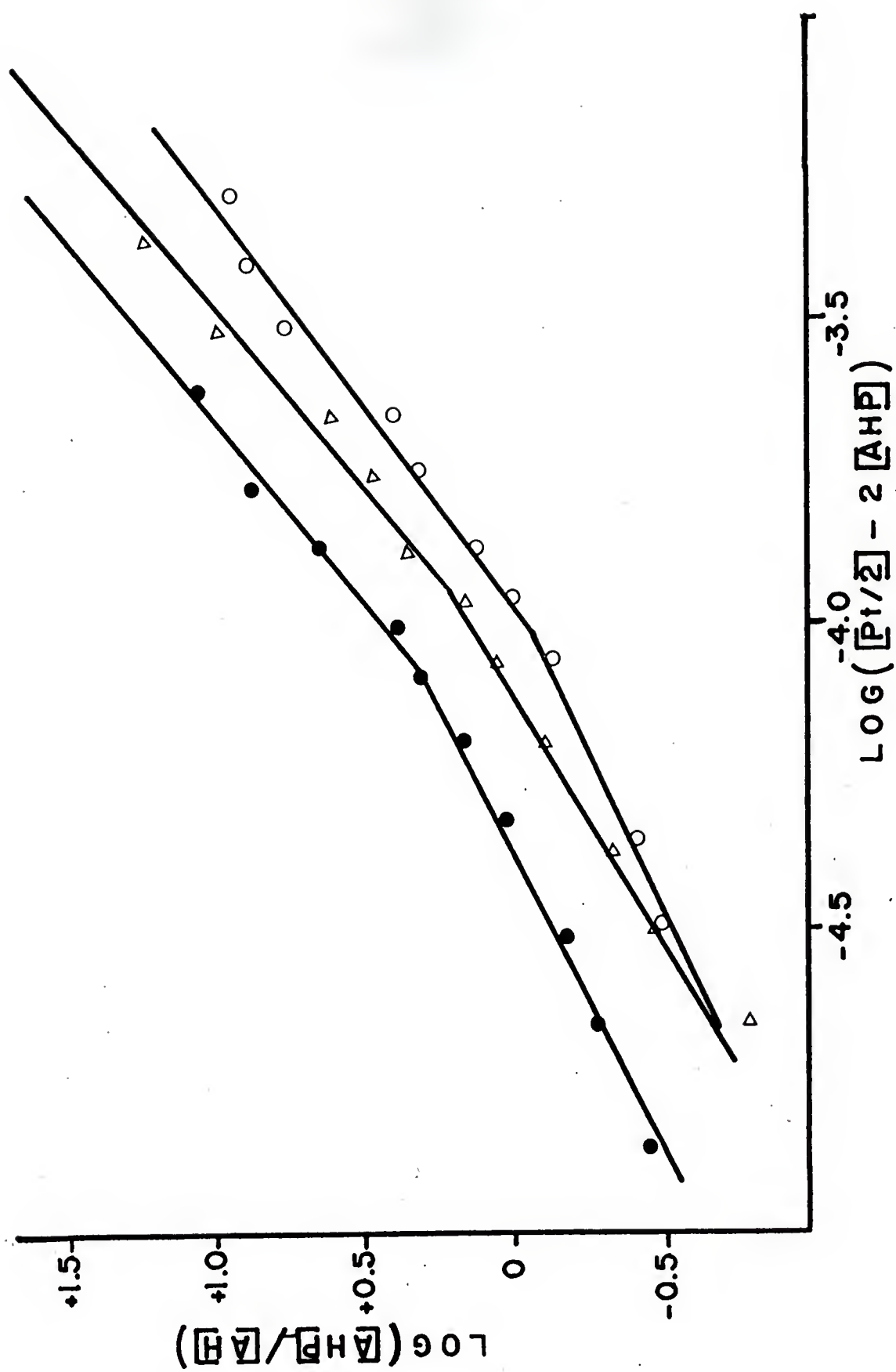


Figure 28.  $\log K_s'$  vs.  $1/T(^{\circ}K)$  for the reaction between 3-aminoacridinium cation and DNA at 15.0°C, 25.0°C, and 35.0°C, pH 5.90, 0.010M  $KH_2PO_4$  as supporting electrolyte



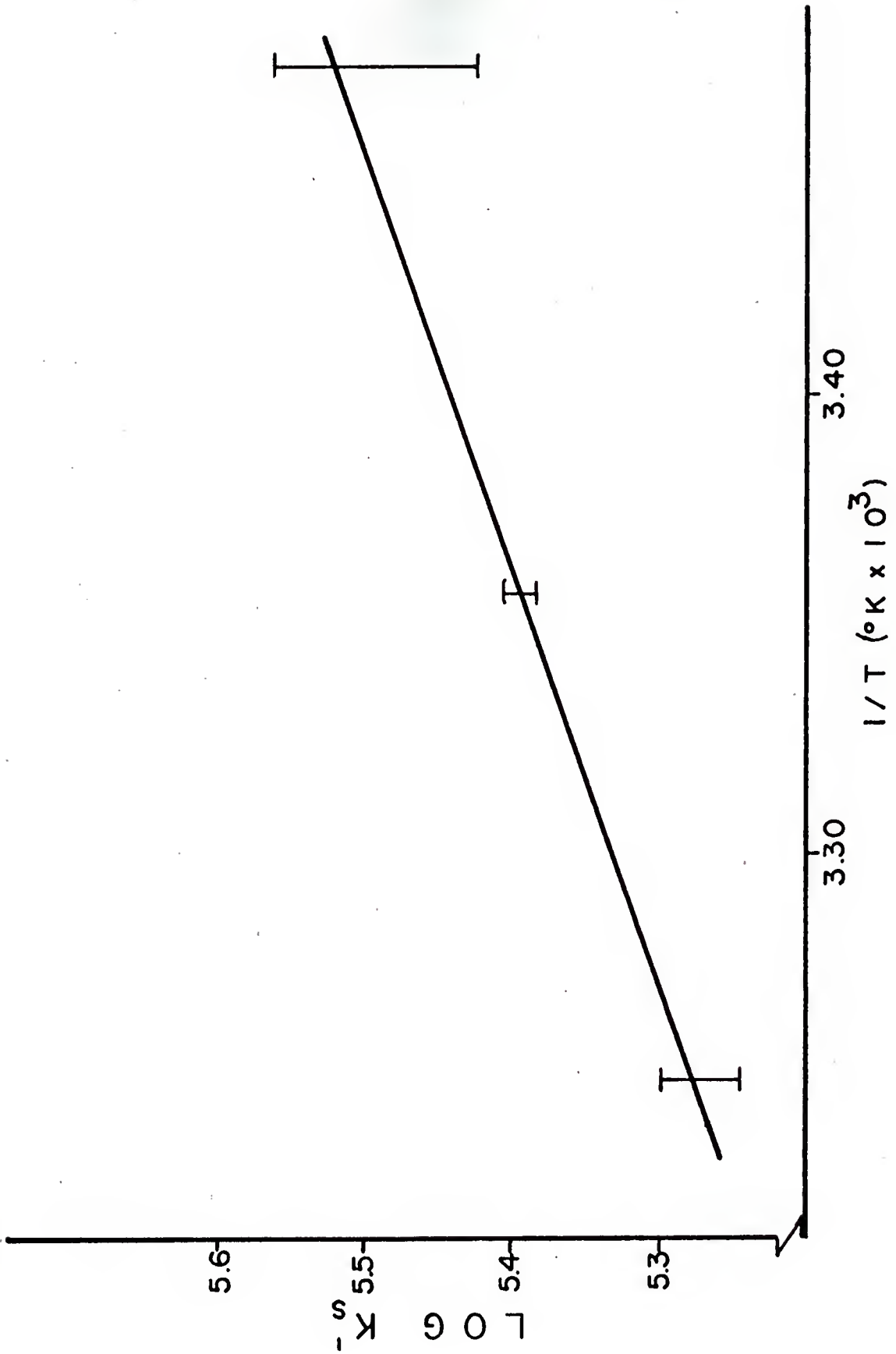


Figure 29.  $\log K_s'$  vs.  $1/T(^{\circ}K)$  for the reaction between 7-aminoquinolinium cation and DNA at 15.0°C, 25.0°C, and 35.0°C, pH 5.90, 0.010M  $KH_2PO_4$  as supporting electrolyte

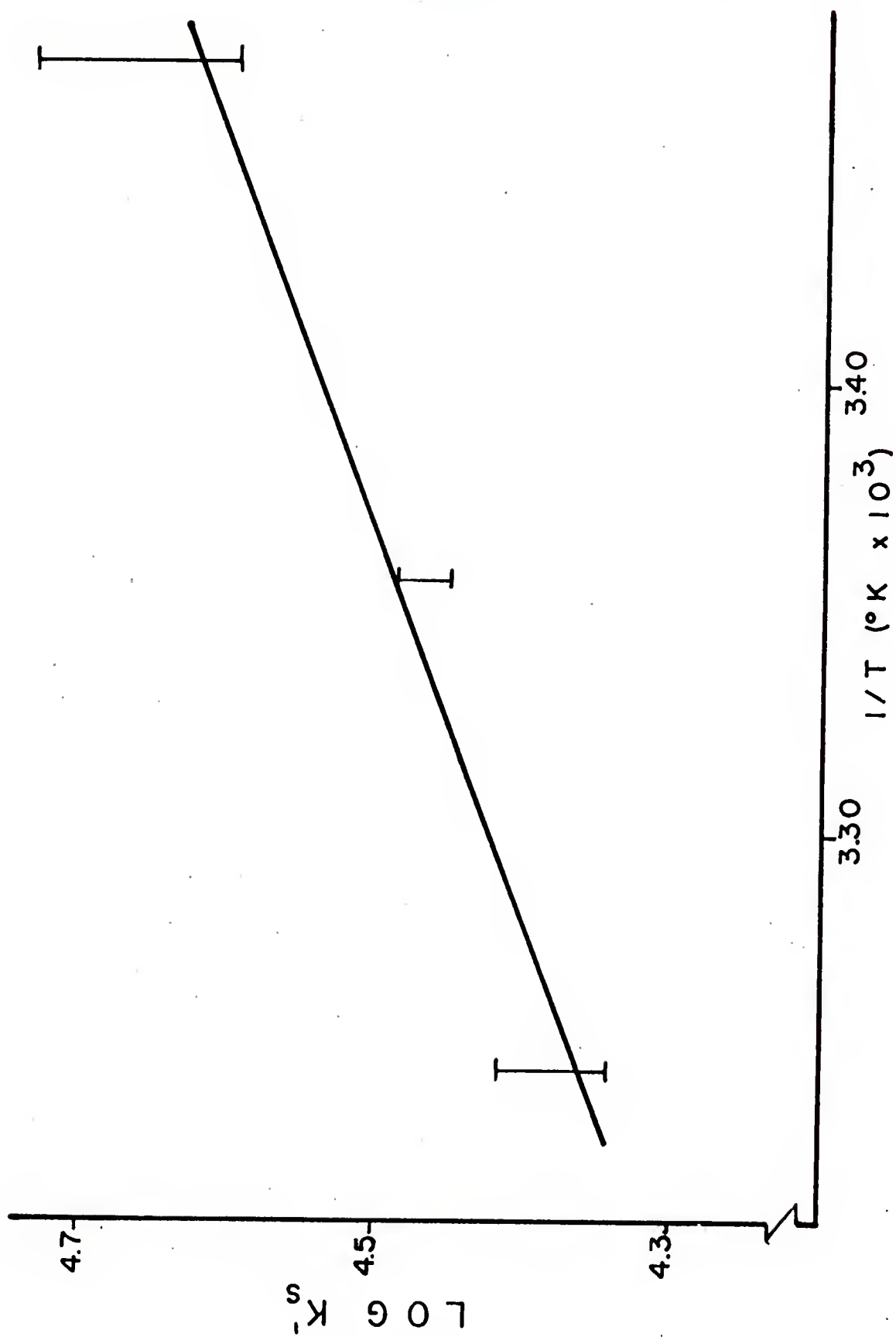


Figure 30.  $\log K_I$  vs.  $1/T(^{\circ}K)$  for the reaction between 3-aminoacridinium cation and DNA at 15.0 $^{\circ}C$ , 25.0 $^{\circ}C$ , and 35.0 $^{\circ}C$ , pH 5.90, 0.010M  $KH_2PO_4$  as supporting electrolyte

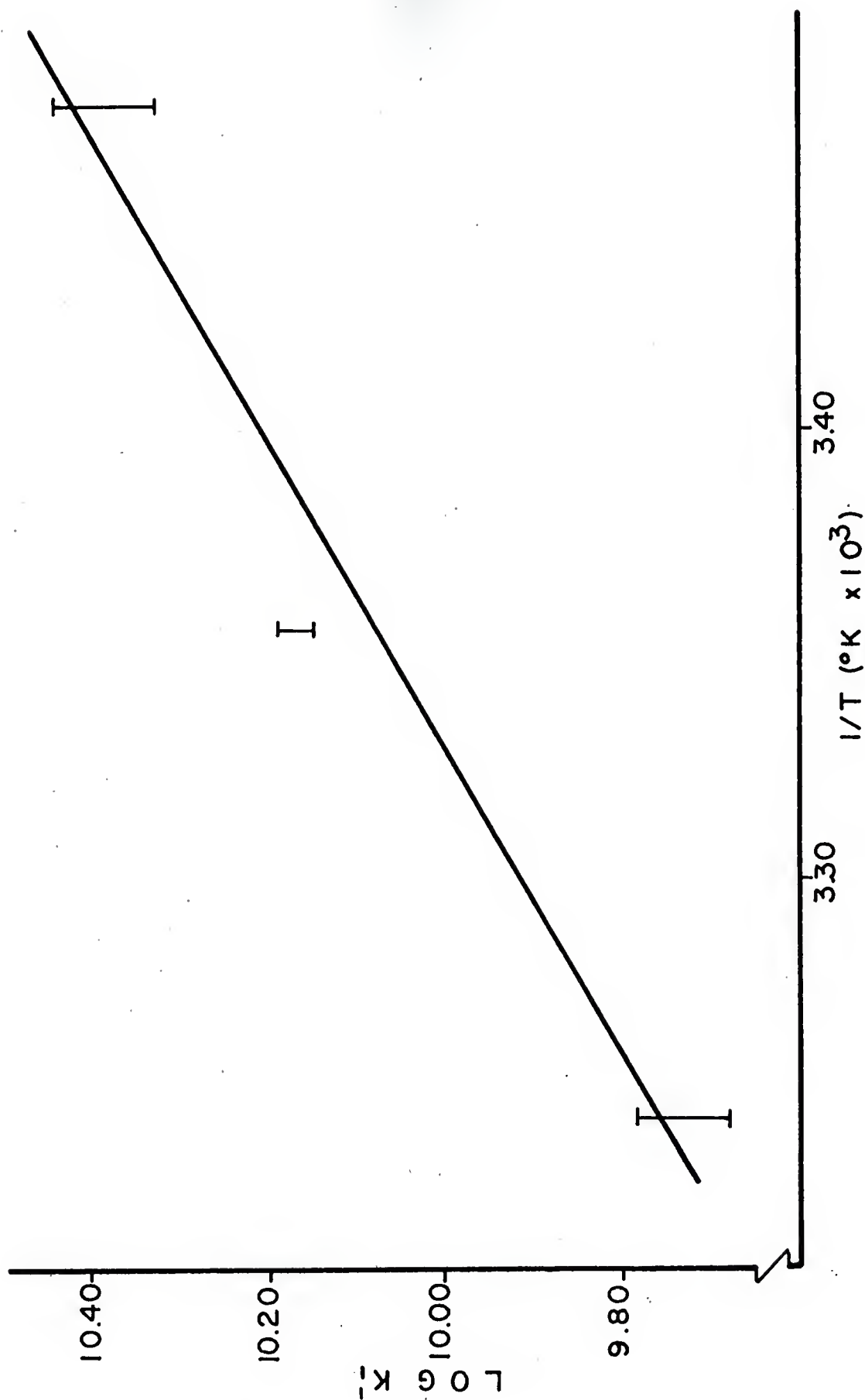
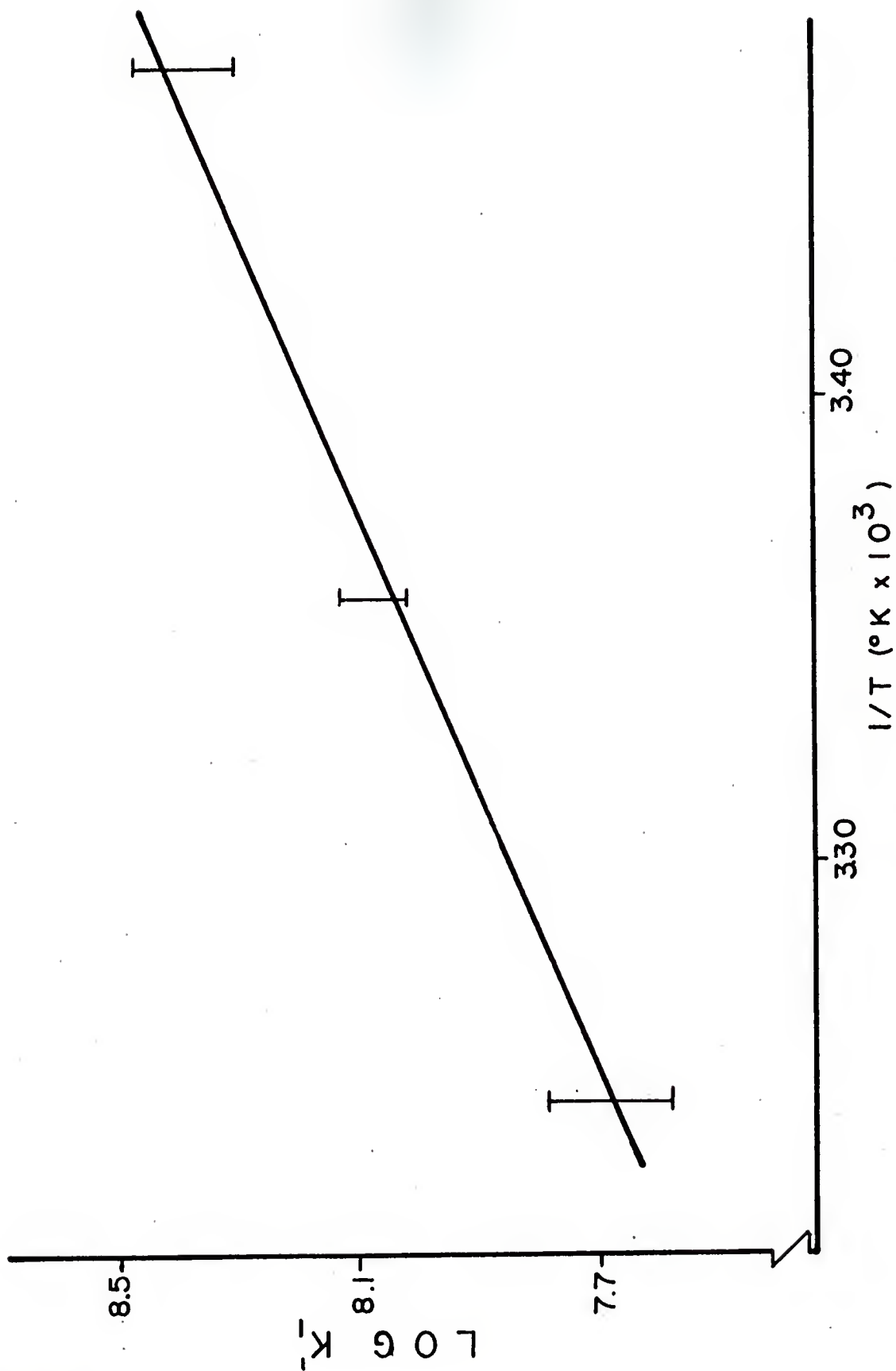


Figure 31.  $\log K_I'$  vs.  $1/T(^{\circ}\text{K})$  for the reaction between 7-aminoquinolinium cation and DNA at 15.0 $^{\circ}\text{C}$ , 25.0 $^{\circ}\text{C}$ , and 35.0 $^{\circ}\text{C}$ , pH 5.90, 0.010M  $\text{KH}_2\text{PO}_4$  as supporting electrolyte



APPENDIX II

TABLES



Table 1. Molar absorptivities of the 3-aminoacridinium-DNA complex and the 7-aminoquinolinium-DNA complex in various supporting electrolytes<sup>a</sup>

| Supporting electrolyte                  | Conc. range of electrolyte (M) | Number of determinations (n) | 3-aminoacridinium-DNA complex                                         |                                              |                                                                       |
|-----------------------------------------|--------------------------------|------------------------------|-----------------------------------------------------------------------|----------------------------------------------|-----------------------------------------------------------------------|
|                                         |                                |                              | $\epsilon_{\text{BHP}} \times 10^{-3} \text{ M}^{-1} \text{ cm}^{-1}$ | Standard deviation $(\sigma) \times 10^{-3}$ | $\epsilon_{\text{BHP}} \times 10^{-3} \text{ M}^{-1} \text{ cm}^{-1}$ |
| $\text{LiH}_2\text{PO}_4$               | .025-.00063                    | 8                            | 8.527                                                                 | 0.111                                        | 9.9097                                                                |
| $\text{NaH}_2\text{PO}_4$               | 0.025-0.0025                   | 6                            | 8.477                                                                 | 0.073                                        | 9.090                                                                 |
| $\text{KH}_2\text{PO}_4$                | 0.025-0.00063                  | 11                           | 8.557                                                                 | 0.082                                        | 9.082                                                                 |
| $\text{KOCCH}_3$                        | 0.025-0.00063                  | 8                            | 8.515                                                                 | 0.059                                        | 9.104                                                                 |
| $\text{RbH}_2\text{PO}_4$               | 0.025-0.0025                   | 10                           | 8.456                                                                 | 0.133                                        | 9.067                                                                 |
| $\text{CsH}_2\text{PO}_4$               | 0.025-0.0025                   | 6                            | 8.763                                                                 | 0.069                                        | 9.353                                                                 |
| $(\text{CH}_3)_4\text{NH}_2\text{PO}_4$ | 0.10-0.0025                    | 18                           | 8.429                                                                 | 0.125                                        | 8.988                                                                 |
|                                         | $\epsilon_{\text{BHP}}$        | 6                            | 8.494                                                                 | 0.048                                        | 9.071                                                                 |
| 7-aminoquinolinium-DNA complex          |                                |                              |                                                                       |                                              |                                                                       |
| $\text{KH}_2\text{PO}_4$                | 0.010                          | 10                           | 5.332                                                                 | 0.094                                        |                                                                       |
|                                         | $\epsilon_{\text{AHP}}$        | 10                           | 5.332                                                                 |                                              |                                                                       |

<sup>a</sup>All at pH 5.90 and 25.0°C. Initial concentration of drug species was 1 to 2 x 10<sup>-5</sup>M.

Table 2 . Absorptiometric titration of 3-aminoacridinium cation with DNA, lithium salt.  
Lithium dihydrogen phosphate as supporting electrolyte <sup>a</sup>

| <u>0.10M<sup>b</sup></u>       |                        |                                |                        | <u>0.05M<sup>b</sup></u>       |                        |                                |                        |
|--------------------------------|------------------------|--------------------------------|------------------------|--------------------------------|------------------------|--------------------------------|------------------------|
| <u>Run 1</u>                   |                        | <u>Run 2</u>                   |                        | <u>Run 1</u>                   |                        | <u>Run 2</u>                   |                        |
| <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>f</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>f</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> |
| 0                              | 0.980                  | 0                              | 0.645                  | 0                              | 0.976                  | 0                              | 0.670                  |
| 10.0                           | 0.957                  | 15.0                           | 0.623                  | 10.0                           | 0.945                  | 10.0                           | 0.662                  |
| 20.0                           | 0.936                  | 30.0**                         | 0.600                  | 20.0**                         | 0.920                  | 20.0                           | 0.640                  |
| 40.0**                         | 0.900                  | 45.0**                         | 0.581                  | 30.0**                         | 0.893                  | 35.0**                         | 0.613                  |
| 60.0**                         | 0.865                  | 65.0**                         | 0.554                  | 40.0**                         | 0.870                  | 50.0**                         | 0.589                  |
| 80.0**                         | 0.835                  | 85.0**                         | 0.538                  | 60.0**                         | 0.822                  | 65.0**                         | 0.567                  |
| 100**                          | 0.809                  | 105                            | 0.517                  | 80.0                           | 0.783                  | 85.0                           | 0.540                  |
| 125                            | 0.779                  | 130                            | 0.497                  | 100                            | 0.750                  | 105                            | 0.518                  |
| 150                            | 0.751                  | 155                            | 0.480                  | 125                            | 0.712                  | 125                            | 0.501                  |
| 200                            | 0.710                  | 180                            | 0.468                  | 150*                           | 0.683                  | 150                            | 0.482                  |
| 250*                           | 0.680                  | 220*                           | 0.452                  | 175*                           | 0.661                  | 175*                           | 0.467                  |
| 300*                           | 0.658                  | 260*                           | 0.438                  | 200*                           | 0.642                  | 210*                           | 0.452                  |
| 400*                           | 0.627                  | 310*                           | 0.426                  | 250*                           | 0.615                  | 250*                           | 0.437                  |
| 500*                           | 0.603                  | 410*                           | 0.410                  | 300                            | 0.600                  | 295*                           | 0.430                  |
| 600                            | 0.591                  | 560                            | 0.399                  | 350                            | 0.588                  | 345                            | 0.423                  |
| 700                            | 0.582                  | 780                            | 0.392                  | 450                            | 0.572                  | 445                            | 0.414                  |
| A <sub>∞</sub> <sup>e</sup>    | 0.551                  | A <sub>∞</sub> <sup>e</sup>    | 0.360                  | A <sub>∞</sub> <sup>e</sup>    | 0.556                  | A <sub>∞</sub> <sup>e</sup>    | 0.375                  |



Table 2 (Continued)

| <u>0.0050M<sup>b</sup></u>     |                        | <u>0.0025M<sup>b</sup></u>     |                        |
|--------------------------------|------------------------|--------------------------------|------------------------|
| <u>Run 2</u>                   |                        | <u>Run 1</u>                   |                        |
| <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> |
| 0                              | 0.973                  | 0                              | 0.990                  |
| 10.0                           | 0.910                  | 10.0                           | 0.925                  |
| 20.0**                         | 0.868                  | 20.0**                         | 0.878                  |
| 30.0**                         | 0.823                  | 30.0**                         | 0.831                  |
| 40.0                           | 0.787                  | 40.0                           | 0.791                  |
| 50.0                           | 0.752                  | 50.0                           | 0.752                  |
| 60.0*                          | 0.721                  | 60.0*                          | 0.719                  |
| 80.0*                          | 0.667                  | 80.0*                          | 0.660                  |
| 100*                           | 0.624                  | 100                            | 0.618                  |
| 125                            | 0.589                  | 125                            | 0.591                  |
| 150                            | 0.571                  | 150                            | 0.581                  |
| 175                            | 0.568                  | 200                            | 0.575                  |
| A <sub>∞</sub> <sup>e</sup>    | 0.575                  | A <sub>∞</sub> <sup>e</sup>    | 0.584                  |
|                                |                        | 110                            | 0.410                  |
|                                |                        | 130                            | 0.400                  |
|                                |                        | 180                            | 0.394                  |
|                                |                        | 280                            | 0.393                  |
|                                |                        | A <sub>∞</sub> <sup>e</sup>    | 0.384                  |
| <u>Run 3</u>                   |                        | <u>Run 2</u>                   |                        |
| <u>Vol<sup>f</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> |
| 0                              | 0.657                  | 0                              | 0.981                  |
| 7.5                            | 0.630                  | 10.0**                         | 0.932                  |
| 15.0**                         | 0.604                  | 20.0**                         | 0.883                  |
| 22.5**                         | 0.583                  | 30.0**                         | 0.840                  |
| 30.0**                         | 0.561                  | 40.0**                         | 0.800                  |
| 37.5                           | 0.542                  | 50.0                           | 0.765                  |
| 45.0                           | 0.523                  | 60.0                           | 0.741                  |
| 55.0                           | 0.499                  | 70.0*                          | 0.707                  |
| 65.0*                          | 0.475                  | 80.0*                          | 0.678                  |
| 75.0*                          | 0.455                  | 90.0*                          | 0.655                  |
| 85.0*                          | 0.441                  | 100*                           | 0.638                  |
| 95.0*                          | 0.429                  | 120                            | 0.610                  |
|                                |                        | 140                            | 0.592                  |
|                                |                        | 190                            | 0.584                  |
|                                |                        | 240                            | 0.579                  |
|                                |                        | A <sub>∞</sub> <sup>e</sup>    | 0.576                  |

Table 2 (Continued)

0.00063M<sup>b</sup>

| <u>Run 1</u>                |                        | <u>Run 2</u>                |                        |
|-----------------------------|------------------------|-----------------------------|------------------------|
| <u>Vol f</u><br><u>DNA</u>  | <u>Abs<sup>d</sup></u> | <u>Vol f</u><br><u>DNA</u>  | <u>Abs<sup>d</sup></u> |
| 0                           | 0.772                  | 0                           | 0.068                  |
| 5.0                         | 0.702                  | 5.0                         | 0.654                  |
| 10.0                        | 0.683                  | 10.0                        | 0.633                  |
| 15.0*                       | 0.662                  | 15.0**                      | 0.615                  |
| 22.5**                      | 0.636                  | 22.5**                      | 0.587                  |
| 30.0**                      | 0.607                  | 30.0**                      | 0.558                  |
| 37.5                        | 0.581                  | 37.5                        | 0.530                  |
| 45.0                        | 0.554                  | 45.0                        | 0.508                  |
| 52.5*                       | 0.530                  | 52.5*                       | 0.483                  |
| 60.0*                       | 0.508                  | 60.0*                       | 0.464                  |
| 70.0*                       | 0.483                  | 70.0*                       | 0.438                  |
| 80.0*                       | 0.464                  | 80.0                        | 0.422                  |
| 90.0                        | 0.451                  | 90.0                        | 0.413                  |
| 110                         | 0.439                  | 110                         | 0.407                  |
| 160                         | 0.435                  | 210                         | 0.405                  |
| 260                         | 0.434                  | 310                         | 0.404                  |
| A <sub>∞</sub> <sup>e</sup> | 0.423                  | A <sub>∞</sub> <sup>e</sup> | 0.397                  |

a<sub>T</sub> = 25.0 ± 0.1°C, pH = 5.90.

Table 2 (Continued)

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|   |                                                                                                                                                                     |
|---|---------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| b | Initial total concentration of supporting electrolyte.                                                                                                              |
| c | Total volume of solution of calf thymus DNA added ( $\mu$ l). Titrant contained $9.300 \times 10^{-9}$ moles of DNA phosphate per microliter.                       |
| d | Total absorbance of sample at 365 nm, 4.00 cm pathlength. Data marked (**) used to compute $K_s$ , those marked (*) were used to compute $K_1$ .                    |
| e | Calculated absorbance of solution if all 3-aminoacridinium cation is bound. Based on molar absorptivity of bound species of $8494 \text{ M}^{-1} \text{ cm}^{-1}$ . |
| f | Same as c above except titrant contained $8.373 \times 10^{-9}$ moles of DNA phosphate per microliter.                                                              |
| g | Same as c above except titrant contained $9.416 \times 10^{-9}$ moles of DNA phosphate per microliter.                                                              |

Table 3 . Absorptiometric titration of 3-aminoacridinium cation with DNA, sodium salt. Sodium dihydrogen phosphate as supporting electrolyte a

| <u>0.100M<sup>b</sup></u>      |                        |                                |                        | <u>0.050M<sup>b</sup></u>      |                        |                                |                        |
|--------------------------------|------------------------|--------------------------------|------------------------|--------------------------------|------------------------|--------------------------------|------------------------|
| <u>Run 1</u>                   |                        | <u>Run 2</u>                   |                        | <u>Run 1</u>                   |                        | <u>Run 2</u>                   |                        |
| <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>d</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> |
| 0                              | 0.940                  | 0                              | 0.953                  | 0                              | 0.948                  | 0                              | 0.961                  |
| 10.0                           | 0.912                  | 10.0                           | 0.929                  | 10.0                           | 0.919                  | 10.0                           | 0.930                  |
| 20.0                           | 0.891                  | 20.0                           | 0.908                  | 20.0                           | 0.889                  | 20.0                           | 0.900                  |
| 30.0**                         | 0.872                  | 40.0**                         | 0.870                  | 30.0**                         | 0.862                  | 30.0**                         | 0.871                  |
| 40.0**                         | 0.851                  | 60.0**                         | 0.832                  | 40.0**                         | 0.835                  | 40.0**                         | 0.848                  |
| 60.0**                         | 0.812                  | 80.0**                         | 0.802                  | 60.0**                         | 0.791                  | 60.0**                         | 0.800                  |
| 80.0**                         | 0.780                  | 100**                          | 0.776                  | 80.0**                         | 0.750                  | 80.0                           | 0.758                  |
| 100                            | 0.752                  | 125                            | 0.745                  | 100                            | 0.713                  | 100                            | 0.720                  |
| 150                            | 0.691                  | 150                            | 0.720                  | 125*                           | 0.677                  | 125*                           | 0.683                  |
| 200*                           | 0.648                  | 175                            | 0.698                  | 150*                           | 0.648                  | 150*                           | 0.652                  |
| 250*                           | 0.619                  | 200*                           | 0.679                  | 200*                           | 0.608                  | 175*                           | 0.637                  |
| 300*                           | 0.602                  | 250*                           | 0.650                  | 250                            | 0.583                  | 200*                           | 0.610                  |
| 400                            | 0.578                  | 300*                           | 0.626                  | 300                            | 0.570                  | 250                            | 0.585                  |
| 500                            | 0.564                  | 350*                           | 0.610                  | 350                            | 0.561                  | 300                            | 0.572                  |
| 600                            | 0.556                  | 400*                           | 0.598                  | 400                            | 0.558                  | 350                            | 0.563                  |
| 700                            | 0.550                  | 500                            | 0.577                  | 500                            | 0.552                  | 400                            | 0.561                  |
| A <sup>e</sup> <sub>∞</sub>    | 0.528                  | 650                            | 0.560                  | 600                            | 0.551                  | 500                            | 0.556                  |
|                                |                        | 800                            | 0.550                  | A <sup>e</sup> <sub>∞</sub>    | 0.536                  | 600                            | 0.555                  |
|                                |                        | 888                            | 0.543                  |                                |                        | A <sup>e</sup> <sub>∞</sub>    | 0.545                  |
|                                |                        | A <sup>e</sup> <sub>∞</sub>    | 0.526                  |                                |                        |                                |                        |

Table 3 (Continued)

| <u>0.025M<sup>b</sup></u>            |                        |                                      |                        | <u>0.010M<sup>b</sup></u>            |                        |                                      |                        |
|--------------------------------------|------------------------|--------------------------------------|------------------------|--------------------------------------|------------------------|--------------------------------------|------------------------|
| <u>Run 1</u>                         |                        | <u>Run 2</u>                         |                        | <u>Run 1</u>                         |                        | <u>Run 2</u>                         |                        |
| <u>Vol<sub>C</sub></u><br><u>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sub>C</sub></u><br><u>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sub>C</sub></u><br><u>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sub>C</sub></u><br><u>DNA</u> | <u>Abs<sup>d</sup></u> |
| 0                                    | 0.958                  | 0                                    | 0.973                  | 0                                    | 0.952                  | 0                                    | 0.955                  |
| 10.0                                 | 0.920                  | 10.0                                 | 0.934                  | 10.0                                 | 0.905                  | 10.0                                 | 0.910                  |
| 20.0**                               | 0.887                  | 20.0**                               | 0.900                  | 20.0**                               | 0.866                  | 20.0**                               | 0.868                  |
| 30.0**                               | 0.853                  | 30.0**                               | 0.868                  | 30.0**                               | 0.829                  | 30.0**                               | 0.831                  |
| 40.0**                               | 0.828                  | 40.0**                               | 0.837                  | 40.0**                               | 0.795                  | 40.0                                 | 0.795                  |
| 60.0                                 | 0.772                  | 60.0                                 | 0.781                  | 60.0                                 | 0.730                  | 60.0**                               | 0.730                  |
| 80.0*                                | 0.722                  | 80.0*                                | 0.730                  | 80.0*                                | 0.675                  | 80.0**                               | 0.675                  |
| 100*                                 | 0.686                  | 100*                                 | 0.689                  | 100*                                 | 0.631                  | 100**                                | 0.631                  |
| 125*                                 | 0.644                  | 125*                                 | 0.650                  | 125                                  | 0.593                  | 125                                  | 0.595                  |
| 150*                                 | 0.615                  | 150*                                 | 0.621                  | 150                                  | 0.572                  | 150                                  | 0.574                  |
| 175                                  | 0.597                  | 175                                  | 0.601                  | 175                                  | 0.562                  | 175                                  | 0.564                  |
| 200                                  | 0.583                  | 200                                  | 0.587                  | 200                                  | 0.558                  | 200                                  | 0.561                  |
| 225                                  | 0.578                  | 225                                  | 0.578                  | 225                                  | 0.558                  | 250                                  | 0.559                  |
| 250                                  | 0.570                  | 250                                  | 0.572                  | 275                                  | 0.552                  | 300                                  | 0.558                  |
| 275                                  | 0.567                  | 300                                  | 0.569                  | <sup>e</sup> A <sub>∞</sub>          | 0.557                  | <sup>e</sup> A <sub>∞</sub>          | 0.558                  |
| 325                                  | 0.564                  | 350                                  | 0.565                  |                                      |                        |                                      |                        |
| 375                                  | 0.560                  | <sup>e</sup> A <sub>∞</sub>          | 0.565                  |                                      |                        |                                      |                        |
| 425                                  | 0.560                  |                                      |                        |                                      |                        |                                      |                        |
| <sup>e</sup> A <sub>∞</sub>          | 0.553                  |                                      |                        |                                      |                        |                                      |                        |



Table 3 (Continued)

| <u>0.0050M<sup>b</sup></u>     |                        | <u>0.0025M</u>                 |                        |
|--------------------------------|------------------------|--------------------------------|------------------------|
| <u>Run 1</u>                   |                        | <u>Run 1</u>                   |                        |
| <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> |
| 0                              | 0.951                  | 0                              | 0.948                  |
| 10.0                           | 0.887                  | 10.0**                         | 0.890                  |
| 20.0**                         | 0.843                  | 20.0**                         | 0.842                  |
| 30.0**                         | 0.800                  | 30.0**                         | 0.800                  |
| 40.0**                         | 0.762                  | 40.0                           | 0.761                  |
| 60.0                           | 0.691                  | 50.0                           | 0.727                  |
| 80.0*                          | 0.634                  | 60.0*                          | 0.694                  |
| 100*                           | 0.597                  | 80.0*                          | 0.636                  |
| 125                            | 0.571                  | 100                            | 0.595                  |
| 150                            | 0.562                  | 125                            | 0.567                  |
| 175                            | 0.560                  | 150                            | 0.559                  |
| 200                            | 0.557                  | 175                            | 0.555                  |
| <sup>e</sup><br>A <sub>∞</sub> | 0.561                  | <sup>e</sup><br>A <sub>∞</sub> | 0.560                  |

<sup>a</sup>T = 25.0 ± 0.1°C, pH = 5.90.

<sup>b</sup>Initial total concentration of supporting electrolyte.

<sup>c</sup>Total volume of solution of calf thymus DNA added (μl). Titrant contained 8.860 × 10<sup>-9</sup> moles of DNA phosphate per microliter.

Table 3 (Continued)

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d Total absorbance of sample at 365 nm, 4.00 cm pathlength. Data points marked (\*\*) used to compute  $K_s$ , those marked (\*) were used to compute  $K_1$ .

e Calculated absorbance of solution if all 3-aminoacridinium cation is bound. Based on molar absorptivity of bound drug of  $8494\text{M}^{-1}\text{cm}^{-1}$ .



Table 4 (Continued)

| <u>.025M<sup>b</sup></u>       |                        |                                |                        | <u>.010M<sup>b</sup></u>       |                        |                                |                        |
|--------------------------------|------------------------|--------------------------------|------------------------|--------------------------------|------------------------|--------------------------------|------------------------|
| <u>Run 1</u>                   |                        | <u>Run 2</u>                   |                        | <u>Run 1</u>                   |                        | <u>Run 2</u>                   |                        |
| <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> |
| 0                              | 0.718                  | 0                              | 0.713                  | 0                              | 0.667                  | 0                              | 0.716                  |
| 10.0                           | 0.687                  | 10.0                           | 0.679                  | 5.0                            | 0.649                  | 7.5                            | 0.687                  |
| 20.0**                         | 0.656                  | 20.0**                         | 0.654                  | 10.0**                         | 0.631                  | 15.0**                         | 0.662                  |
| 30.0**                         | 0.630                  | 30.0**                         | 0.627                  | 17.5**                         | 0.606                  | 22.5**                         | 0.635                  |
| 40.0**                         | 0.605                  | 40.0**                         | 0.605                  | 25.0**                         | 0.583                  | 30.0**                         | 0.614                  |
| 50.0                           | 0.584                  | 50.0                           | 0.586                  | 32.5**                         | 0.562                  | 40.0                           | 0.587                  |
| 60.0                           | 0.565                  | 65.0                           | 0.554                  | 40.0                           | 0.542                  | 50.0                           | 0.563                  |
| 70.0                           | 0.546                  | 80.0*                          | 0.528                  | 50.0                           | 0.517                  | 60.0                           | 0.537                  |
| 85.0*                          | 0.519                  | 95.0*                          | 0.504                  | 60.0*                          | 0.497                  | 70.0*                          | 0.516                  |
| 100*                           | 0.498                  | 110*                           | 0.486                  | 70.0*                          | 0.477                  | 80.0*                          | 0.496                  |
| 115*                           | 0.480                  | 125*                           | 0.470                  | 80.0*                          | 0.460                  | 90.0*                          | 0.480                  |
| 130*                           | 0.467                  | 145*                           | 0.452                  | 90.0*                          | 0.443                  | 100*                           | 0.467                  |
| 150*                           | 0.452                  | 170                            | 0.442                  | 100*                           | 0.431                  | 115                            | 0.450                  |
| 175                            | 0.441                  | 220                            | 0.425                  | 115*                           | 0.419                  | 135                            | 0.435                  |
| 200                            | 0.435                  | 270                            | 0.418                  | 130                            | 0.410                  | 160                            | 0.426                  |
| 300                            | 0.427                  | 370                            | 0.416                  | 180                            | 0.399                  | 200                            | 0.422                  |
| 400                            | 0.425                  | A <sub>∞</sub> <sup>e</sup>    | 0.414                  | 280                            | 0.396                  | 300                            | 0.420                  |
| A <sub>∞</sub> <sup>e</sup>    | 0.415                  |                                |                        | A <sub>∞</sub> <sup>e</sup>    | 0.390                  | A <sub>∞</sub> <sup>e</sup>    | 0.418                  |

Table 4 (Continued)

| <u>0.0050M<sup>b</sup></u>  |                  |                             |                  | <u>0.00063M<sup>b</sup></u> |                  |                |                  |
|-----------------------------|------------------|-----------------------------|------------------|-----------------------------|------------------|----------------|------------------|
| Run 1                       |                  | Run 2                       |                  | Run 1                       |                  | Run 2          |                  |
| Vol C<br>DNA                | Abs <sup>d</sup> | Vol C<br>DNA                | Abs <sup>d</sup> | Vol C<br>DNA                | Abs <sup>d</sup> | Vol C<br>DNA   | Abs <sup>d</sup> |
| 0                           | 0.714            | 0                           | 0.666            | 0                           | 0.669            | 0              | 0.698            |
| 5.0                         | 0.697            | 7.5                         | 0.627            | 5.0                         | 0.637            | 5.0            | 0.668            |
| 10.0**                      | 0.679            | 15.0**                      | 0.600            | 10.0**                      | 0.612            | 10.0**         | 0.649            |
| 15.0**                      | 0.664            | 22.5**                      | 0.578            | 15.0**                      | 0.593            | 15.0**         | 0.629            |
| 22.5**                      | 0.641            | 30.0                        | 0.554            | 20.0**                      | 0.576            | 20.0**         | 0.612            |
| 30.0**                      | 0.618            | 37.5                        | 0.532            | 27.5**                      | 0.548            | 27.5**         | 0.583            |
| 40.0                        | 0.592            | 45.0                        | 0.512            | 35.0                        | 0.521            | 35.0           | 0.560            |
| 50.0                        | 0.565            | 52.5                        | 0.497            | 42.5                        | 0.500            | 42.5           | 0.535            |
| 60.0                        | 0.547            | 60.0*                       | 0.479            | 50.0*                       | 0.477            | 50.0*          | 0.510            |
| 70.0*                       | 0.522            | 70.0*                       | 0.460            | 57.5*                       | 0.454            | 57.5*          | 0.489            |
| 85.0*                       | 0.496            | 80.0*                       | 0.443            | 65.0*                       | 0.439            | 65.0*          | 0.469            |
| 100*                        | 0.470            | 90.0*                       | 0.428            | 75.0                        | 0.421            | 75.0*          | 0.449            |
| 115*                        | 0.453            | 110                         | 0.407            | 85.0                        | 0.410            | 85.0           | 0.431            |
| 130                         | 0.442            | 130                         | 0.397            | 100                         | 0.407            | 95.0           | 0.423            |
| 155                         | 0.434            | 170                         | 0.389            | 125                         | 0.405            | 115            | 0.416            |
| 230                         | 0.426            | 270                         | 0.388            | A <sub>∞</sub> <sup>e</sup> | 0.397            | 165            | 0.415            |
| 330                         | 0.424            | A <sub>∞</sub> <sup>e</sup> | 0.390            |                             |                  | A <sup>e</sup> | 0.413            |
| A <sub>∞</sub> <sup>e</sup> | 0.416            |                             |                  |                             |                  |                |                  |

<sup>a</sup>T = 25.0 ± 0.1°C, pH = 5.90.

Table 4 (Continued)

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|                                                                                                                                                                              |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <sup>b</sup> Initial total concentration of supporting electrolyte.                                                                                                          |
| <sup>c</sup> Total volume of solution of calf thymus DNA added ( $\mu$ l). Titrant contained $8.09 \times 10^{-9}$ moles of DNA phosphate per microliter.                    |
| <sup>d</sup> Total absorbance of sample at 365 nm, 4.00 cm pathlength. Data marked (**) used to compute $K_s$ , those marked (*) were used to compute $K_1$ .                |
| <sup>e</sup> Calculated absorbance of solution if all 3-aminoacridinium cation is bound. Based on molar absorptivity of bound species of $8494\text{M}^{-1}\text{cm}^{-1}$ . |

Table 5 . Absorptiometric titration of 3-aminoacridinium cation with DNA, potassium salt. Potassium acetate as supporting electrolyte <sup>a</sup>

| <u>0.15M<sup>b</sup></u>    |                  | <u>0.050M<sup>b</sup></u>   |                  |
|-----------------------------|------------------|-----------------------------|------------------|
| Run 1                       |                  | Run 1                       |                  |
| Vol <sup>c</sup><br>DNA     | Abs <sup>d</sup> | Vol <sup>c</sup><br>DNA     | Abs <sup>d</sup> |
| 0                           | 0.721            | 0                           | 0.724            |
| 10.0                        | 0.706            | 10.0                        | 0.698            |
| 20.0                        | 0.690            | 20.0**                      | 0.677            |
| 40.0**                      | 0.662            | 30.0**                      | 0.653            |
| 60.0**                      | 0.638            | 40.0**                      | 0.631            |
| 80.0**                      | 0.606            | 50.0**                      | 0.611            |
| 100**                       | 0.597            | 60.0                        | 0.593            |
| 125                         | 0.572            | 70.0                        | 0.577            |
| 150                         | 0.553            | 85.0                        | 0.553            |
| 175                         | 0.537            | 100                         | 0.533            |
| 200                         | 0.523            | 115*                        | 0.516            |
| 250*                        | 0.500            | 130*                        | 0.500            |
| 300*                        | 0.483            | 150*                        | 0.485            |
| 350*                        | 0.467            | 175*                        | 0.467            |
| 400*                        | 0.458            | 200*                        | 0.463            |
| 450*                        | 0.449            | 225                         | 0.455            |
| 550*                        | 0.437            | 275                         | 0.448            |
| 650                         | 0.429            | 325                         | 0.437            |
| 850                         | 0.422            | 425                         | 0.430            |
| 1050                        | 0.415            | 625                         | 0.423            |
| 1300                        | 0.414            | A <sup>e</sup> <sub>∞</sub> | 0.422            |
| A <sup>e</sup> <sub>∞</sub> | 0.384            |                             | 0.410            |
| Run 2                       |                  | Run 2                       |                  |
| Vol <sup>c</sup><br>DNA     | Abs <sup>d</sup> | Vol <sup>c</sup><br>DNA     | Abs <sup>d</sup> |
| 0                           | 0.747            | 0                           | 0.731            |
| 20.0                        | 0.716            | 10.0                        | 0.705            |
| 40.0**                      | 0.686            | 20.0**                      | 0.683            |
| 60.0**                      | 0.659            | 30.0**                      | 0.663            |
| 80.0**                      | 0.638            | 40.0**                      | 0.642            |
| 100**                       | 0.619            | 50.0**                      | 0.623            |
| 125                         | 0.597            | 65.0                        | 0.594            |
| 150                         | 0.578            | 85.0                        | 0.571            |
| 175                         | 0.562            | 95.0                        | 0.549            |
| 200*                        | 0.547            | 110*                        | 0.531            |
| 250*                        | 0.522            | 130*                        | 0.510            |
| 300*                        | 0.505            | 150*                        | 0.493            |
| 350*                        | 0.492            | 175*                        | 0.477            |
| 450                         | 0.470            | 200*                        | 0.463            |
| 550                         | 0.459            | 225                         | 0.454            |
| 700                         | 0.449            | 275                         | 0.443            |
| 900                         | 0.441            | 375                         | 0.433            |
| A <sup>e</sup> <sub>∞</sub> | 0.412            | 575                         | 0.427            |
|                             |                  | A <sup>e</sup> <sub>∞</sub> | 0.416            |

Table 5 (Continued)

| <u>0.025M<sup>b</sup></u>            |                        |                                      |                        | <u>0.0050M<sup>b</sup></u>           |                        |                                      |                        |
|--------------------------------------|------------------------|--------------------------------------|------------------------|--------------------------------------|------------------------|--------------------------------------|------------------------|
| <u>Run 1</u>                         |                        | <u>Run 2</u>                         |                        | <u>Run 1</u>                         |                        | <u>Run 2</u>                         |                        |
| <u>Vol<sub>C</sub></u><br><u>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sub>C</sub></u><br><u>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sub>C</sub></u><br><u>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sub>C</sub></u><br><u>DNA</u> | <u>Abs<sup>d</sup></u> |
| 0                                    | 0.713                  | 0                                    | 0.749                  | 0                                    | 0.704                  | 0                                    | 0.739                  |
| 10.0                                 | 0.684                  | 10.0                                 | 0.718                  | 10.0**                               | 0.656                  | 7.5                                  | 0.695                  |
| 20.0**                               | 0.655                  | 20.0**                               | 0.693                  | 15.0**                               | 0.636                  | 15.0                                 | 0.670                  |
| 35.0**                               | 0.613                  | 30.0**                               | 0.668                  | 20.0**                               | 0.619                  | 22.5**                               | 0.648                  |
| 50.0**                               | 0.578                  | 40.0**                               | 0.644                  | 27.5**                               | 0.592                  | 30.0**                               | 0.620                  |
| 65.0                                 | 0.546                  | 50.0                                 | 0.622                  | 35.0                                 | 0.569                  | 37.5                                 | 0.600                  |
| 80.0*                                | 0.517                  | 65.0                                 | 0.591                  | 42.5                                 | 0.547                  | 45.0                                 | 0.581                  |
| 95.0*                                | 0.496                  | 80.0                                 | 0.565                  | 50.0                                 | 0.527                  | 55.0                                 | 0.552                  |
| 110*                                 | 0.477                  | 95.0*                                | 0.542                  | 60.0*                                | 0.501                  | 65.0*                                | 0.529                  |
| 125*                                 | 0.461                  | 110*                                 | 0.523                  | 70.0*                                | 0.480                  | 75.0*                                | 0.513                  |
| 145*                                 | 0.445                  | 130*                                 | 0.504                  | 80.0*                                | 0.465                  | 85.0*                                | 0.496                  |
| 165                                  | 0.433                  | 150*                                 | 0.487                  | 90.0*                                | 0.450                  | A <sup>e</sup> <sub>∞</sub>          | 0.441                  |
| 185                                  | 0.426                  | 175*                                 | 0.474                  | 100                                  | 0.439                  |                                      |                        |
| 210                                  | 0.420                  | 210                                  | 0.461                  | 120                                  | 0.424                  |                                      |                        |
| 260                                  | 0.414                  | 260                                  | 0.449                  | 145                                  | 0.418                  |                                      |                        |
| 310                                  | 0.411                  | 360                                  | 0.438                  | 190                                  | 0.419                  |                                      |                        |
| 510                                  | 0.409                  | A <sup>e</sup> <sub>∞</sub>          | 0.435                  | A <sup>e</sup> <sub>∞</sub>          | 0.415                  |                                      |                        |
| A <sup>e</sup> <sub>∞</sub>          | 0.408                  |                                      |                        |                                      |                        |                                      |                        |



Table 5 (Continued)

| <u>0.0050M<sup>b</sup></u>     |                        |  | <u>0.0025M<sup>b</sup></u>     |                        |  |
|--------------------------------|------------------------|--|--------------------------------|------------------------|--|
| <u>Run 3</u>                   |                        |  | <u>Run 1</u>                   |                        |  |
| <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> |  | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> |  |
| 0                              | 0.720                  |  | 0                              | 0.721                  |  |
| 7.5                            | 0.689                  |  | 5.0                            | 0.700                  |  |
| 15.0**                         | 0.662                  |  | 10.0**                         | 0.679                  |  |
| 22.5**                         | 0.637                  |  | 17.5**                         | 0.649                  |  |
| 30.0**                         | 0.610                  |  | 25.0**                         | 0.623                  |  |
| 37.5                           | 0.588                  |  | 32.5**                         | 0.598                  |  |
| 45.0                           | 0.565                  |  | 40.0                           | 0.572                  |  |
| 52.5                           | 0.547                  |  | 47.5                           | 0.550                  |  |
| 60.0*                          | 0.526                  |  | 55.0*                          | 0.528                  |  |
| 70.0*                          | 0.504                  |  | 62.5*                          | 0.508                  |  |
| 80.0*                          | 0.485                  |  | 70.0*                          | 0.490                  |  |
| 90.0*                          | 0.466                  |  | 80.0*                          | 0.469                  |  |
| 100                            | 0.454                  |  | 90.0                           | 0.452                  |  |
| 110                            | 0.447                  |  | 100                            | 0.440                  |  |
| 140                            | 0.432                  |  | 110                            | 0.432                  |  |
| 240                            | 0.429                  |  | 130                            | 0.424                  |  |
| A <sub>∞</sub> <sup>e</sup>    | 0.423                  |  | 180                            | 0.422                  |  |
|                                |                        |  | A <sub>∞</sub> <sup>e</sup>    | 0.426                  |  |
| <u>Run 2</u>                   |                        |  | <u>Run 2</u>                   |                        |  |
| <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> |  | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> |  |
| 0                              | 0.727                  |  | 0                              | 0.727                  |  |
| 7.5                            | 0.691                  |  | 7.5                            | 0.691                  |  |
| 15.0**                         | 0.665                  |  | 15.0**                         | 0.665                  |  |
| 22.5**                         | 0.637                  |  | 22.5**                         | 0.637                  |  |
| 30.0                           | 0.612                  |  | 30.0                           | 0.612                  |  |
| 37.5                           | 0.588                  |  | 37.5                           | 0.588                  |  |
| 45.0                           | 0.564                  |  | 45.0                           | 0.564                  |  |
| 55.0*                          | 0.533                  |  | 55.0*                          | 0.533                  |  |
| 65.0*                          | 0.509                  |  | 65.0*                          | 0.509                  |  |
| 75.0*                          | 0.486                  |  | 75.0*                          | 0.486                  |  |
| 85.0*                          | 0.467                  |  | 85.0*                          | 0.467                  |  |
| 95.0                           | 0.453                  |  | 95.0                           | 0.453                  |  |
| 105                            | 0.443                  |  | 105                            | 0.443                  |  |
| 130                            | 0.430                  |  | 130                            | 0.430                  |  |
| 180                            | 0.429                  |  | 180                            | 0.429                  |  |
| A <sub>∞</sub> <sup>e</sup>    | 0.429                  |  | A <sub>∞</sub> <sup>e</sup>    | 0.429                  |  |

Table 5 (Continued)

| <u>0.00063M<sup>b</sup></u>    |                        |                                |                        |
|--------------------------------|------------------------|--------------------------------|------------------------|
| <u>Run 1</u>                   |                        | <u>Run 2</u>                   |                        |
| <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>d</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> |
| 0                              | 0.715                  | 0                              | 0.713                  |
| 5.0                            | 0.693                  | 7.5                            | 0.688                  |
| 10.0**                         | 0.675                  | 15.0**                         | 0.665                  |
| 15.0**                         | 0.656                  | 22.5**                         | 0.640                  |
| 20.0**                         | 0.638                  | 30.0**                         | 0.617                  |
| 27.5**                         | 0.610                  | 37.5                           | 0.593                  |
| 35.0**                         | 0.587                  | 45.0                           | 0.573                  |
| 42.5                           | 0.560                  | 55.0                           | 0.543                  |
| 50.0                           | 0.536                  | 65.0*                          | 0.517                  |
| 57.5*                          | 0.514                  | 75.0*                          | 0.496                  |
| 65.0*                          | 0.495                  | 85.0*                          | 0.474                  |
| 75.0*                          | 0.468                  | 105                            | 0.442                  |
| 85.0*                          | 0.449                  | 125                            | 0.429                  |
| 95.0                           | 0.437                  | 150                            | 0.427                  |
| 115                            | 0.427                  | A <sup>e</sup> <sub>∞</sub>    | 0.422                  |
| 165                            | 0.424                  |                                |                        |
| 265                            | 0.424                  |                                |                        |
| A <sup>e</sup> <sub>∞</sub>    | 0.419                  |                                |                        |

<sup>a</sup>T = 25.0 ± 0.1°C, pH = 5.90.

Table 5 (Continued)

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|                                                                                                                                                                              |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <sup>b</sup> Initial total concentration of supporting electrolyte.                                                                                                          |
| <sup>c</sup> Total volume of solution of calf thymus DNA added ( $\mu$ l). Titrant contained $8.163 \times 10^{-9}$ moles of DNA phosphate per microliter.                   |
| <sup>d</sup> Total absorbance of sample at 365 nm, 4.00 cm pathlength. Data marked (**) used to compute $K_s$ , those marked (*) were used to compute $K_1$ .                |
| <sup>e</sup> Calculated absorbance of solution if all 3-aminoacridinium cation is bound. Based on molar absorptivity of bound species of $8494\text{M}^{-1}\text{cm}^{-1}$ . |

Table 6 . Absorptiometric titration of 3-aminoacridinium cation with DNA, rubidium salt. Rubidium dihydrogen phosphate as supporting electrolyte a

| <u>0.10M<sup>b</sup></u>       |                        | <u>0.050M<sup>b</sup></u>      |                        |
|--------------------------------|------------------------|--------------------------------|------------------------|
| <u>Run 1</u>                   |                        | <u>Run 1</u>                   |                        |
| <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> |
| 0                              | 0.760                  | 0                              | 0.720                  |
| 20.0**                         | 0.723                  | 10.0                           | 0.698                  |
| 40.0**                         | 0.691                  | 20.0**                         | 0.678                  |
| 60.0**                         | 0.662                  | 40.0**                         | 0.638                  |
| 80.0**                         | 0.637                  | 60.0**                         | 0.603                  |
| 100                            | 0.613                  | 80.0                           | 0.571                  |
| 125                            | 0.588                  | 100                            | 0.546                  |
| 150                            | 0.569                  | 120                            | 0.525                  |
| 175                            | 0.550                  | 140*                           | 0.506                  |
| 200*                           | 0.535                  | 160*                           | 0.490                  |
| 225*                           | 0.526                  | 180*                           | 0.478                  |
| 250*                           | 0.515                  | 205*                           | 0.465                  |
| 300*                           | 0.498                  | 255                            | 0.455                  |
| 350*                           | 0.485                  | 280                            | 0.450                  |
| 400*                           | 0.476                  | 305                            | 0.438                  |
| 500                            | 0.462                  | 405                            | 0.427                  |
| 600                            | 0.456                  | 505                            | 0.422                  |
| 700                            | 0.450                  | A <sub>∞</sub> <sup>e</sup>    | 0.412                  |
| A <sub>∞</sub> <sup>e</sup>    | 0.427                  |                                |                        |
| <u>Run 2</u>                   |                        | <u>Run 2</u>                   |                        |
| <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> |
| 0                              | 0.738                  | 0                              | 0.748                  |
| 20.0**                         | 0.705                  | 25.0**                         | 0.689                  |
| 40.0**                         | 0.673                  | 50.0**                         | 0.641                  |
| 60.0**                         | 0.643                  | 75.0                           | 0.598                  |
| 80.0**                         | 0.617                  | 100                            | 0.564                  |
| 100                            | 0.595                  | 125*                           | 0.536                  |
| 125                            | 0.569                  | 150*                           | 0.513                  |
| 150                            | 0.548                  | 175*                           | 0.495                  |
| 175*                           | 0.531                  | 200*                           | 0.482                  |
| 200*                           | 0.517                  | 215*                           | 0.478                  |
| 225*                           | 0.502                  | 230*                           | 0.471                  |
| 250*                           | 0.493                  | 250                            | 0.464                  |
| 275*                           | 0.484                  | 270                            | 0.459                  |
| 300*                           | 0.480                  | 295                            | 0.454                  |
| 350*                           | 0.467                  | 345                            | 0.447                  |
| 400*                           | 0.458                  | 445                            | 0.439                  |
| 450                            | 0.450                  | 545                            | 0.435                  |
| 550                            | 0.440                  | A <sub>∞</sub> <sup>e</sup>    | 0.423                  |
| 650                            | 0.437                  |                                |                        |
| 750                            | 0.432                  |                                |                        |
| A <sub>∞</sub> <sup>e</sup>    | 0.413                  |                                |                        |

Table 6 (Continued)

| <u>0.025M<sup>b</sup></u>      |                        | <u>0.010M<sup>b</sup></u>      |                        |
|--------------------------------|------------------------|--------------------------------|------------------------|
| <u>Run 1</u>                   |                        | <u>Run 3</u>                   |                        |
| <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> |
| 0                              | 0.778                  | 0                              | 0.719                  |
| 10.0                           | 0.744                  | 20.0**                         | 0.662                  |
| 20.0**                         | 0.719                  | 40.0**                         | 0.613                  |
| 30.0**                         | 0.695                  | 60.0                           | 0.571                  |
| 40.0**                         | 0.673                  | 80.0                           | 0.536                  |
| 60.0                           | 0.630                  | 100*                           | 0.506                  |
| 80.0                           | 0.593                  | 115*                           | 0.490                  |
| 100*                           | 0.561                  | 130*                           | 0.476                  |
| 120*                           | 0.535                  | 145*                           | 0.462                  |
| 140*                           | 0.517                  | 160                            | 0.454                  |
| 160*                           | 0.499                  | 175                            | 0.445                  |
| 185*                           | 0.485                  | 200                            | 0.435                  |
| 210                            | 0.475                  | 225                            | 0.429                  |
| 260                            | 0.462                  | 275                            | 0.422                  |
| 310                            | 0.458                  | 350                            | 0.418                  |
| 410                            | 0.454                  | A <sub>∞</sub> <sup>e</sup>    | 0.418                  |
| A <sub>∞</sub> <sup>e</sup>    | 0.445                  |                                |                        |
| <u>Run 2</u>                   |                        | <u>Run 1</u>                   |                        |
| <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> |
| 0                              | 0.735                  | 0                              | 0.701                  |
| 20.0**                         | 0.674                  | 10.0                           | 0.673                  |
| 40.0**                         | 0.627                  | 20.0**                         | 0.644                  |
| 60.0                           | 0.585                  | 30.0**                         | 0.613                  |
| 80.0                           | 0.549                  | 40.0                           | 0.585                  |
| 100*                           | 0.520                  | 60.0                           | 0.537                  |
| 115*                           | 0.502                  | 80.0*                          | 0.496                  |
| 130*                           | 0.483                  | 100*                           | 0.467                  |
| 145*                           | 0.474                  | 120*                           | 0.444                  |
| 160*                           | 0.463                  | 140                            | 0.431                  |
| 175                            | 0.457                  | 160                            | 0.420                  |
| 200                            | 0.445                  | 210                            | 0.413                  |
| 225                            | 0.440                  | 260                            | 0.411                  |
| 275                            | 0.437                  | A <sub>∞</sub> <sup>e</sup>    | 0.411                  |
| 350                            | 0.432                  |                                |                        |
| A <sub>∞</sub> <sup>e</sup>    | 0.427                  |                                |                        |



Table 6 (Continued)

0.0025M<sup>b</sup>

| <u>Run 1</u>                   |                        | <u>Run 2</u>                   |                        |
|--------------------------------|------------------------|--------------------------------|------------------------|
| <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> |
| 0                              | 0.752                  | 0                              | 0.725                  |
| 10.0                           | 0.714                  | 10.0                           | 0.688                  |
| 20.0**                         | 0.683                  | 20.0**                         | 0.656                  |
| 30.0**                         | 0.653                  | 30.0**                         | 0.627                  |
| 40.0**                         | 0.624                  | 40.0**                         | 0.600                  |
| 50.0                           | 0.597                  | 50.0                           | 0.572                  |
| 60.0                           | 0.574                  | 60.0                           | 0.549                  |
| 70.0*                          | 0.550                  | 70.0*                          | 0.528                  |
| 80.0*                          | 0.531                  | 80.0*                          | 0.508                  |
| 90.0*                          | 0.510                  | 90.0*                          | 0.491                  |
| 110*                           | 0.479                  | 100*                           | 0.478                  |
| 130                            | 0.459                  | 110*                           | 0.465                  |
| 150                            | 0.446                  | 120                            | 0.455                  |
| 175                            | 0.439                  | 135                            | 0.443                  |
| 225                            | 0.431                  | 150                            | 0.436                  |
| A <sub>∞</sub> <sup>e</sup>    | 0.442                  | 175                            | 0.430                  |
|                                |                        | 225                            | 0.428                  |
|                                |                        | A <sub>∞</sub> <sup>e</sup>    | 0.426                  |

<sup>a</sup>T = 25.0 ± 0.1°C, pH = 5.90.

Table 6 (Continued)

- 
- <sup>b</sup>Initial total concentration of supporting electrolyte.
- <sup>c</sup>Total volume of solution of calf thymus DNA added ( $\mu$ l). Titrant contained  $7.209 \times 10^{-9}$  moles of DNA phosphate per microliter.
- <sup>d</sup>Total absorbance of sample at 365 nm, 4.00 cm pathlength. Data marked (\*\*) used to compute  $K_s$ , those marked (\*) were used to compute  $K_1$ .
- <sup>e</sup>Calculated absorbance of solution if all 3-aminoacridinium cation is bound. Based on molar absorptivity of bound species of  $8494 \text{ M}^{-1} \text{ cm}^{-1}$ .



Table 7 . Absorptiometric titration of 3-aminoacridinium cation with DNA, cesium salt.  
Cesium dihydrogen phosphate as supporting electrolyte <sup>a</sup>

| <u>0.15M<sup>b</sup></u>       |                        |                                |                        |
|--------------------------------|------------------------|--------------------------------|------------------------|
| <u>Run 1</u>                   |                        | <u>Run 2</u>                   |                        |
| <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> |
| 0                              | 0.868                  | 0                              | 0.763                  |
| 10.0                           | 0.844                  | 15.0                           | 0.738                  |
| 25.0**                         | 0.829                  | 30.0**                         | 0.716                  |
| 40.0**                         | 0.803                  | 45.0**                         | 0.692                  |
| 55.0**                         | 0.783                  | 60.0**                         | 0.672                  |
| 70.0**                         | 0.763                  | 75.0**                         | 0.654                  |
| 85.0**                         | 0.747                  | 90.0**                         | 0.635                  |
| 100**                          | 0.729                  | 110**                          | 0.613                  |
| 120                            | 0.708                  | 130                            | 0.596                  |
| 140                            | 0.685                  | 150                            | 0.582                  |
| 160                            | 0.669                  | 170                            | 0.570                  |
| 180                            | 0.654                  | 195                            | 0.552                  |
| 205                            | 0.633                  | 225*                           | 0.540                  |
| 230*                           | 0.622                  | 265*                           | 0.521                  |
| 280*                           | 0.596                  | 315*                           | 0.507                  |
| 330*                           | 0.578                  | 365*                           | 0.497                  |
| 380*                           | 0.564                  | 415*                           | 0.488                  |
| 480*                           | 0.544                  | 515*                           | 0.474                  |
| 580*                           | 0.533                  | 715*                           | 0.460                  |
| 760                            | 0.525                  | 1015                           | 0.454                  |
| A <sub>∞</sub> <sup>e</sup>    | 0.485                  | A <sub>∞</sub> <sup>e</sup>    | 0.417                  |

| <u>0.10M<sup>b</sup></u>       |                        |                                |                        |
|--------------------------------|------------------------|--------------------------------|------------------------|
| <u>Run 1</u>                   |                        | <u>Run 2</u>                   |                        |
| <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> |
| 0                              | 0.782                  | 0                              | 0.749                  |
| 20.0**                         | 0.742                  | 10.0                           | 0.715                  |
| 35.0**                         | 0.713                  | 20.0**                         | 0.691                  |
| 50.0**                         | 0.687                  | 30.0**                         | 0.676                  |
| 60.0**                         | 0.673                  | 40.0**                         | 0.659                  |
| 70.0**                         | 0.661                  | 50.0**                         | 0.640                  |
| 85.0*                          | 0.639                  | 65.0                           | 0.621                  |
| 100                            | 0.627                  | 80.0                           | 0.597                  |
| 120                            | 0.600                  | 95.0                           | 0.581                  |
| 140                            | 0.584                  | 110                            | 0.565                  |
| 160                            | 0.569                  | 130                            | 0.545                  |
| 185*                           | 0.550                  | 150                            | 0.530                  |
| 210*                           | 0.539                  | 170*                           | 0.516                  |
| 235*                           | 0.527                  | 205*                           | 0.501                  |
| 285*                           | 0.508                  | 240*                           | 0.485                  |
| 335*                           | 0.498                  | 290*                           | 0.469                  |
| 385*                           | 0.488                  | 340                            | 0.458                  |
| 485                            | 0.479                  | 440                            | 0.443                  |
| 785                            | 0.471                  | 640                            | 0.437                  |
| A <sub>∞</sub> <sup>e</sup>    | 0.436                  | 840                            | 0.436                  |
|                                |                        | A <sub>∞</sub> <sup>e</sup>    | 0.416                  |

Table 7 (Continued)

| <u>0.050M<sup>b</sup></u>   |                        | <u>0.025M<sup>b</sup></u>   |                        |
|-----------------------------|------------------------|-----------------------------|------------------------|
| <u>Run 1</u>                |                        | <u>Run 1</u>                |                        |
| <u>Vol C</u><br><u>DNA</u>  | <u>Abs<sup>d</sup></u> | <u>Vol C</u><br><u>DNA</u>  | <u>Abs<sup>d</sup></u> |
| 0                           | 0.763                  | 0                           | 0.767                  |
| 5.0                         | 0.752                  | 7.5                         | 0.734                  |
| 10.0                        | 0.742                  | 15.0**                      | 0.713                  |
| 17.5                        | 0.727                  | 22.5**                      | 0.692                  |
| 25.0**                      | 0.712                  | 30.0**                      | 0.670                  |
| 32.5**                      | 0.697                  | 37.5**                      | 0.651                  |
| 40.0**                      | 0.677                  | 45.0**                      | 0.630                  |
| 50.0**                      | 0.660                  | 55.0                        | 0.610                  |
| 60.0**                      | 0.640                  | 65.0                        | 0.587                  |
| 70.0                        | 0.627                  | 75.0                        | 0.570                  |
| 85.0                        | 0.600                  | 90.0*                       | 0.545                  |
| 100                         | 0.581                  | 105*                        | 0.520                  |
| 115                         | 0.562                  | 120*                        | 0.506                  |
| 130                         | 0.548                  | 135*                        | 0.490                  |
| 145*                        | 0.533                  | 155*                        | 0.477                  |
| 165*                        | 0.519                  | 180                         | 0.466                  |
| 190*                        | 0.505                  | 230                         | 0.453                  |
| 240*                        | 0.485                  | 330                         | 0.451                  |
| 290*                        | 0.477                  | 630                         | 0.450                  |
| 390                         | 0.467                  | A <sub>∞</sub> <sup>e</sup> | 0.434                  |
| 590                         | 0.462                  |                             |                        |
| A <sub>∞</sub> <sup>e</sup> | 0.433                  |                             |                        |
| <u>Run 2</u>                |                        | <u>Run 2</u>                |                        |
| <u>Vol C</u><br><u>DNA</u>  | <u>Abs<sup>d</sup></u> | <u>Vol C</u><br><u>DNA</u>  | <u>Abs<sup>d</sup></u> |
| 0                           | 0.740                  | 0                           | 0.740                  |
| 7.5                         | 0.714                  | 7.5                         | 0.714                  |
| 15.0**                      | 0.699                  | 15.0**                      | 0.699                  |
| 22.5**                      | 0.679                  | 22.5**                      | 0.679                  |
| 30.0**                      | 0.659                  | 30.0**                      | 0.659                  |
| 37.5**                      | 0.648                  | 37.5**                      | 0.648                  |
| 45.0**                      | 0.631                  | 45.0**                      | 0.631                  |
| 55.0**                      | 0.612                  | 55.0**                      | 0.612                  |
| 65.0                        | 0.594                  | 65.0                        | 0.594                  |
| 75.0                        | 0.580                  | 75.0                        | 0.580                  |
| 85.0                        | 0.560                  | 85.0                        | 0.560                  |
| 100                         | 0.542                  | 100                         | 0.542                  |
| 115*                        | 0.528                  | 115*                        | 0.528                  |
| 130*                        | 0.511                  | 130*                        | 0.511                  |
| 150*                        | 0.495                  | 150*                        | 0.495                  |
| 170*                        | 0.479                  | 170*                        | 0.479                  |
| 195*                        | 0.470                  | 195*                        | 0.470                  |
| 230                         | 0.460                  | 230                         | 0.460                  |
| 280                         | 0.448                  | 280                         | 0.448                  |
| 380                         | 0.442                  | 380                         | 0.442                  |
| 580                         | 0.441                  | 580                         | 0.441                  |
| A <sub>∞</sub> <sup>e</sup> | 0.421                  | A <sub>∞</sub> <sup>e</sup> | 0.421                  |
| <u>Run 1</u>                |                        | <u>Run 2</u>                |                        |
| <u>Vol C</u><br><u>DNA</u>  | <u>Abs<sup>d</sup></u> | <u>Vol C</u><br><u>DNA</u>  | <u>Abs<sup>d</sup></u> |
| 0                           | 0.778                  | 0                           | 0.778                  |
| 7.5                         | 0.752                  | 7.5                         | 0.752                  |
| 15.0**                      | 0.733                  | 15.0**                      | 0.733                  |
| 22.5**                      | 0.719                  | 22.5**                      | 0.719                  |
| 30.0**                      | 0.702                  | 30.0**                      | 0.702                  |
| 40.0**                      | 0.680                  | 40.0**                      | 0.680                  |
| 50.0**                      | 0.661                  | 50.0**                      | 0.661                  |
| 65.0                        | 0.645                  | 65.0                        | 0.645                  |
| 75.0                        | 0.619                  | 75.0                        | 0.619                  |
| 90.0                        | 0.594                  | 90.0                        | 0.594                  |
| 105                         | 0.577                  | 105                         | 0.577                  |
| 120*                        | 0.558                  | 120*                        | 0.558                  |
| 140*                        | 0.539                  | 140*                        | 0.539                  |
| 160*                        | 0.522                  | 160*                        | 0.522                  |
| 185*                        | 0.504                  | 185*                        | 0.504                  |
| 210*                        | 0.494                  | 210*                        | 0.494                  |
| 245                         | 0.483                  | 245                         | 0.483                  |
| 295                         | 0.470                  | 295                         | 0.470                  |
| 395                         | 0.464                  | 395                         | 0.464                  |
| 695                         | 0.457                  | 695                         | 0.457                  |
| A <sub>∞</sub> <sup>e</sup> | 0.437                  | A <sub>∞</sub> <sup>e</sup> | 0.437                  |

Table 7 (Continued)

| <u>0.010M<sup>b</sup></u>      |                        |                                |                        | <u>0.0025M<sup>b</sup></u>     |                        |                                |                        |
|--------------------------------|------------------------|--------------------------------|------------------------|--------------------------------|------------------------|--------------------------------|------------------------|
| <u>Run 1</u>                   |                        | <u>Run 2</u>                   |                        | <u>Run 1</u>                   |                        | <u>Run 2</u>                   |                        |
| <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> |
| 0                              | 0.749                  | 0                              | 0.778                  | 0                              | 0.752                  | 0                              | 0.773                  |
| 5.0                            | 0.727                  | 5.0                            | 0.746                  | 5.0                            | 0.728                  | 5.0                            | 0.739                  |
| 10.0**                         | 0.710                  | 10.0                           | 0.730                  | 10.0                           | 0.701                  | 10.0                           | 0.719                  |
| 17.5**                         | 0.683                  | 15.0**                         | 0.713                  | 15.0**                         | 0.681                  | 15.0**                         | 0.701                  |
| 25.0**                         | 0.662                  | 22.5**                         | 0.691                  | 20.0**                         | 0.666                  | 20.0**                         | 0.686                  |
| 32.5**                         | 0.638                  | 30.0**                         | 0.669                  | 25.0**                         | 0.649                  | 25.0**                         | 0.672                  |
| 40.0**                         | 0.620                  | 37.5**                         | 0.648                  | 30.0**                         | 0.631                  | 32.5**                         | 0.648                  |
| 47.5                           | 0.598                  | 45.0                           | 0.629                  | 37.5                           | 0.605                  | 40.0                           | 0.626                  |
| 55.0                           | 0.580                  | 52.5                           | 0.608                  | 45.0                           | 0.581                  | 47.5                           | 0.604                  |
| 65.0*                          | 0.557                  | 60.0*                          | 0.590                  | 52.5*                          | 0.561                  | 55.0                           | 0.584                  |
| 75.0*                          | 0.539                  | 70.0*                          | 0.572                  | 60.0*                          | 0.539                  | 62.5*                          | 0.569                  |
| 85.0*                          | 0.518                  | 80.0*                          | 0.552                  | 70.0*                          | 0.515                  | 70.0*                          | 0.551                  |
| 100*                           | 0.496                  | 90.0*                          | 0.530                  | 80.0*                          | 0.497                  | 80.0*                          | 0.534                  |
| 120*                           | 0.478                  | 105*                           | 0.510                  | 90.0*                          | 0.481                  | 90.0*                          | 0.518                  |
| 140                            | 0.467                  | 120*                           | 0.493                  | 100                            | 0.467                  | 100*                           | 0.506                  |
| 165                            | 0.458                  | 140                            | 0.477                  | 110                            | 0.462                  | 130                            | 0.475                  |
| 265                            | 0.453                  | 190                            | 0.461                  | 160                            | 0.456                  | 230                            | 0.466                  |
| A <sub>∞</sub> <sup>e</sup>    | 0.439                  | 290                            | 0.455                  | 210                            | 0.453                  | A <sub>∞</sub> <sup>e</sup>    | 0.454                  |
|                                |                        | 590                            | 0.454                  | A <sub>∞</sub> <sup>e</sup>    | 0.443                  |                                |                        |
|                                |                        | A <sub>∞</sub> <sup>e</sup>    | 0.442                  |                                |                        |                                |                        |

Table 7 (Continued)

- 
- <sup>a</sup> $T = 25.0 \pm 0.1^{\circ}\text{C}$ ,  $\text{pH} = 5.90$ .
- <sup>b</sup>Initial total concentration of supporting electrolyte.
- <sup>c</sup>Total volume of solution of calf thymus DNA added ( $\mu\text{l}$ ). Titrant contained  $8.680 \times 10^{-9}$  moles of DNA phosphate per microliter.
- <sup>d</sup>Total absorbance of sample at 365 nm, 4.00 cm pathlength. Data marked (\*\*) used to compute  $K_s$ , those marked (\*) were used to compute  $K_1$ .
- <sup>e</sup>Calculated absorbance of solution if all 3-aminoacridinium cation is bound. Based on molar absorptivity of bound species of  $8494\text{M}^{-1}\text{cm}^{-1}$ .



Table 8 (Continued)

| <u>.0250M<sup>b</sup></u>      |                        |                                |                        |                                |                        |                                |                        |  |  |
|--------------------------------|------------------------|--------------------------------|------------------------|--------------------------------|------------------------|--------------------------------|------------------------|--|--|
| <u>Run 1</u>                   |                        | <u>Run 2</u>                   |                        | <u>Run 3</u>                   |                        | <u>Run 4</u>                   |                        |  |  |
| <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> |  |  |
| 0                              | 0.778                  | 0                              | 0.794                  | 0                              | 0.749                  | 0                              | 0.762                  |  |  |
| 20.0                           | 0.692                  | 10.0**                         | 0.758                  | 20.0**                         | 0.671                  | 10.0**                         | 0.723                  |  |  |
| 40.0**                         | 0.637                  | 20.0**                         | 0.727                  | 40.0**                         | 0.613                  | 20.0**                         | 0.692                  |  |  |
| 60.0**                         | 0.588                  | 30.0**                         | 0.697                  | 60.0                           | 0.548                  | 30.0**                         | 0.663                  |  |  |
| 70.0                           | 0.569                  | 40.0**                         | 0.669                  | 70.0                           | 0.529                  | 40.0                           | 0.636                  |  |  |
| 80.0                           | 0.550                  | 60.0                           | 0.613                  | 80.0                           | 0.513                  | 60.0                           | 0.583                  |  |  |
| 90.0*                          | 0.532                  | 80.0*                          | 0.569                  | 90.0*                          | 0.498                  | 80.0*                          | 0.541                  |  |  |
| 100*                           | 0.520                  | 100*                           | 0.533                  | 95.0*                          | 0.495                  | 100*                           | 0.508                  |  |  |
| 115*                           | 0.500                  | 120*                           | 0.506                  | 100*                           | 0.489                  | 120*                           | 0.481                  |  |  |
| 130*                           | 0.486                  | 140                            | 0.488                  | 105*                           | 0.485                  | 140                            | 0.465                  |  |  |
| 150                            | 0.473                  | 160                            | 0.478                  | 115                            | 0.474                  | 160                            | 0.453                  |  |  |
| 200                            | 0.458                  | 180                            | 0.470                  | 125                            | 0.467                  | 180                            | 0.449                  |  |  |
| 250                            | 0.456                  | 200                            | 0.465                  | 140                            | 0.457                  | 200                            | 0.445                  |  |  |
| A <sub>∞</sub> <sup>e</sup>    | 0.456                  | 250                            | 0.462                  | 155                            | 0.450                  | 250                            | 0.441                  |  |  |
|                                |                        | A <sub>∞</sub> <sup>e</sup>    | 0.466                  | 180                            | 0.442                  | A <sub>∞</sub> <sup>e</sup>    | 0.447                  |  |  |
|                                |                        |                                |                        | A <sub>∞</sub> <sup>e</sup>    | 0.442                  |                                |                        |  |  |

Table 8 (Continued)

| <u>0.010M<sup>b</sup></u>      |                        |                                |                        |                                |                        |
|--------------------------------|------------------------|--------------------------------|------------------------|--------------------------------|------------------------|
| <u>Run 1</u>                   |                        | <u>Run 2</u>                   |                        | <u>Run 3</u>                   |                        |
| <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> |
| 0                              | 1.010                  | 0                              | 0.672                  | 0                              | 0.768                  |
| 10.0**                         | 0.963                  | 10.0**                         | 0.630                  | 20.0**                         | 0.681                  |
| 20.0**                         | 0.922                  | 20.0**                         | 0.592                  | 40.0**                         | 0.608                  |
| 30.0**                         | 0.879                  | 30.0                           | 0.553                  | 60.0*                          | 0.549                  |
| 40.0                           | 0.840                  | 40.0                           | 0.523                  | 70.0*                          | 0.527                  |
| 60.0                           | 0.772                  | 60.0*                          | 0.468                  | 80.0*                          | 0.507                  |
| 80.0*                          | 0.711                  | 80.0*                          | 0.429                  | 90.0*                          | 0.490                  |
| 100*                           | 0.668                  | 100                            | 0.405                  | 100                            | 0.475                  |
| 120*                           | 0.634                  | 120                            | 0.397                  | 110                            | 0.465                  |
| 140                            | 0.610                  | 140                            | 0.390                  | 130                            | 0.454                  |
| 160                            | 0.598                  | 160                            | 0.389                  | 155                            | 0.450                  |
| 200                            | 0.589                  | A <sub>∞</sub> <sup>e</sup>    | 0.398                  | 205                            | 0.449                  |
| 250                            | 0.587                  |                                |                        | A <sub>∞</sub> <sup>e</sup>    | 0.453                  |
| A <sub>∞</sub> <sup>e</sup>    | 0.593                  |                                |                        |                                |                        |

Table 8 (Continued)

0.0050M<sup>b</sup>

| Run 1                       |                  | Run 2                       |                  | Run 3                       |                  |
|-----------------------------|------------------|-----------------------------|------------------|-----------------------------|------------------|
| Vol <sup>c</sup><br>DNA     | Abs <sup>d</sup> | Vol <sup>c</sup><br>DNA     | Abs <sup>d</sup> | Vol <sup>c</sup><br>DNA     | Abs <sup>d</sup> |
| 0                           | 0.758            | 0                           | 0.756            | 0                           | 0.764            |
| 10.0                        | 0.699            | 20.0                        | 0.649            | 10.0**                      | 0.713            |
| 20.0**                      | 0.660            | 40.0                        | 0.571            | 30.0**                      | 0.629            |
| 30.0**                      | 0.622            | 50.0                        | 0.540            | 40.0                        | 0.593            |
| 40.0**                      | 0.588            | 60.0*                       | 0.518            | 50.0                        | 0.558            |
| 50.0                        | 0.558            | 65.0*                       | 0.507            | 60.0*                       | 0.531            |
| 60.0*                       | 0.531            | 70.0*                       | 0.499            | 65.0*                       | 0.524            |
| 80.0*                       | 0.488            | 75.0*                       | 0.489            | 70.0*                       | 0.512            |
| 100                         | 0.462            | 85.0                        | 0.473            | 80.0*                       | 0.493            |
| 125                         | 0.449            | 95.0                        | 0.461            | 90.0                        | 0.476            |
| 150                         | 0.445            | 115                         | 0.445            | 100                         | 0.462            |
| A <sub>∞</sub> <sup>e</sup> | 0.449            | 140                         | 0.439            | 115                         | 0.450            |
|                             |                  | A <sub>∞</sub> <sup>e</sup> | 0.448            | 140                         | 0.445            |
|                             |                  |                             |                  | 165                         | 0.442            |
|                             |                  |                             |                  | A <sub>∞</sub> <sup>e</sup> | 0.452            |



Table 8 (Continued)

| 0.0025M <sup>b</sup>        |                  |                             |                  |                             |                  |
|-----------------------------|------------------|-----------------------------|------------------|-----------------------------|------------------|
| Run 1                       |                  | Run 2                       |                  | Run 3                       |                  |
| Vol <sup>c</sup><br>DNA     | Abs <sup>d</sup> | Vol <sup>c</sup><br>DNA     | Abs <sup>d</sup> | Vol <sup>c</sup><br>DNA     | Abs <sup>d</sup> |
| 0                           | 0.768            | 0                           | 0.768            | 0                           | 0.780            |
| 10.0**                      | 0.719            | 10.0**                      | 0.703            | 10.0**                      | 0.719            |
| 20.0**                      | 0.678            | 30.0**                      | 0.619            | 30.0**                      | 0.640            |
| 30.0**                      | 0.638            | 40.0                        | 0.583            | 40.0                        | 0.605            |
| 40.0                        | 0.600            | 50.0*                       | 0.552            | 50.0                        | 0.573            |
| 50.0*                       | 0.568            | 60.0*                       | 0.524            | 60.0*                       | 0.545            |
| 60.0*                       | 0.537            | 70.0*                       | 0.503            | 70.0*                       | 0.522            |
| 70.0*                       | 0.512            | 75.0*                       | 0.494            | 75.0*                       | 0.515            |
| 80.0*                       | 0.491            | 80.0                        | 0.487            | 80.0*                       | 0.505            |
| 100                         | 0.462            | 90.0                        | 0.471            | 85.0                        | 0.500            |
| 120                         | 0.451            | 100                         | 0.459            | 90.0                        | 0.492            |
| 140                         | 0.446            | 120                         | 0.447            | 100                         | 0.479            |
| A <sub>∞</sub> <sup>e</sup> | 0.456            | 170                         | 0.443            | 125                         | 0.460            |
|                             |                  | A <sub>∞</sub> <sup>e</sup> | 0.454            | 150                         | 0.456            |
|                             |                  |                             |                  | A <sub>∞</sub> <sup>e</sup> | 0.462            |

<sup>a</sup>T = 25.0 ± 0.1°C, pH = 5.90.

<sup>b</sup>Initial total concentration of supporting electrolyte.

Table 8 (Continued)

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|   |                                                                                                                                                                     |
|---|---------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| c | Total volume of solution of calf thymus DNA added ( $\mu$ l). Titrant contained $8.924 \times 10^{-9}$ moles of DNA phosphate per microliter.                       |
| d | Total absorbance of sample at 365 nm, 4.00 cm pathlength. Data marked (**) used to compute $K_s$ , those marked (*) were used to compute $K_1$ .                    |
| e | Calculated absorbance of solution if all 3-aminoacridinium cation is bound. Based on molar absorptivity of bound species of $8494 \text{ M}^{-1} \text{ cm}^{-1}$ . |

Table 9 . Absorptiometric titration of 3-aminoacridinium cation with DNA, magnesium salt. Magnesium acetate as supporting electrolyte a

| <u>0.010N<sup>b</sup></u>      |                        |                                |                        |
|--------------------------------|------------------------|--------------------------------|------------------------|
| <u>.0050N<sup>b</sup></u>      |                        |                                |                        |
| <u>Run 1</u>                   |                        | <u>Run 2</u>                   |                        |
| <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> |
| 0                              | 0.752                  | 0                              | 0.742                  |
| 25.0                           | 0.727                  | 25.0                           | 0.716                  |
| 50.0                           | 0.703                  | 50.0                           | 0.693                  |
| 75.0**                         | 0.685                  | 75.0**                         | 0.673                  |
| 100**                          | 0.664                  | 100**                          | 0.657                  |
| 150**                          | 0.637                  | 150**                          | 0.625                  |
| 200**                          | 0.611                  | 200**                          | 0.600                  |
| 250**                          | 0.590                  | 250                            | 0.581                  |
| 300                            | 0.575                  | 300                            | 0.565                  |
| 350                            | 0.560                  | 350                            | 0.551                  |
| 400                            | 0.548                  | 400                            | 0.541                  |
| 500                            | 0.527                  | 500                            | 0.520                  |
| 600                            | 0.513                  | 600                            | 0.506                  |
| 700                            | 0.500                  | 700                            | 0.495                  |
| 800                            | 0.490                  | 800                            | 0.485                  |
| 1000                           | 0.476                  | 900                            | 0.478                  |
| 1300                           | 0.463                  | 1100                           | 0.467                  |
| 1500                           | 0.460                  | 1300                           | 0.459                  |
| A <sub>∞</sub> <sup>e</sup>    | 0.393                  | 1650                           | 0.449                  |
|                                |                        | A <sub>∞</sub> <sup>e</sup>    | 0.383                  |
| <u>Run 1</u>                   |                        | <u>Run 2</u>                   |                        |
| <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> |
| 0                              | 0.748                  | 0                              | 0.748                  |
| 10.0                           | 0.729                  | 10.0                           | 0.729                  |
| 20.0                           | 0.714                  | 20.0                           | 0.714                  |
| 35.0                           | 0.698                  | 35.0                           | 0.698                  |
| 50.0**                         | 0.679                  | 50.0**                         | 0.679                  |
| 70.0**                         | 0.661                  | 70.0**                         | 0.661                  |
| 90.0**                         | 0.642                  | 90.0**                         | 0.642                  |
| 115**                          | 0.622                  | 115**                          | 0.622                  |
| 140                            | 0.606                  | 140                            | 0.606                  |
| 165                            | 0.591                  | 165                            | 0.591                  |
| 215                            | 0.567                  | 215                            | 0.567                  |
| 265                            | 0.547                  | 265                            | 0.547                  |
| 315                            | 0.530                  | 315                            | 0.530                  |
| 365                            | 0.517                  | 365                            | 0.517                  |
| 415                            | 0.505                  | 415                            | 0.505                  |
| 465                            | 0.490                  | 465                            | 0.490                  |
| 565                            | 0.478                  | 565                            | 0.478                  |
| 665                            | 0.467                  | 665                            | 0.467                  |
| 765                            | 0.459                  | 765                            | 0.459                  |
| 965                            | 0.446                  | 965                            | 0.446                  |
| 1165                           | 0.440                  | 1165                           | 0.440                  |
| 1465                           | 0.430                  | 1465                           | 0.430                  |
| A <sub>∞</sub> <sup>e</sup>    | 0.392                  | A <sub>∞</sub> <sup>e</sup>    | 0.392                  |
| <u>Run 1</u>                   |                        | <u>Run 2</u>                   |                        |
| <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> |
| 0                              | 0.739                  | 0                              | 0.739                  |
| 20.0                           | 0.709                  | 20.0                           | 0.709                  |
| 40.0                           | 0.686                  | 40.0                           | 0.686                  |
| 60.0**                         | 0.669                  | 60.0**                         | 0.669                  |
| 80.0**                         | 0.649                  | 80.0**                         | 0.649                  |
| 100**                          | 0.630                  | 100**                          | 0.630                  |
| 125**                          | 0.613                  | 125**                          | 0.613                  |
| 150                            | 0.597                  | 150                            | 0.597                  |
| 175                            | 0.568                  | 175                            | 0.568                  |
| 200                            | 0.549                  | 200                            | 0.549                  |
| 250                            | 0.530                  | 250                            | 0.530                  |
| 300                            | 0.516                  | 300                            | 0.516                  |
| 350                            | 0.505                  | 350                            | 0.505                  |
| 400                            | 0.496                  | 400                            | 0.496                  |
| 450                            | 0.489                  | 450                            | 0.489                  |
| 550                            | 0.475                  | 550                            | 0.475                  |
| 650                            | 0.466                  | 650                            | 0.466                  |
| 750                            | 0.458                  | 750                            | 0.458                  |
| 950                            | 0.447                  | 950                            | 0.447                  |
| A <sub>∞</sub> <sup>e</sup>    | 0.406                  | A <sub>∞</sub> <sup>e</sup>    | 0.406                  |

Table 9 (Continued)

| <u>0.0025N<sup>b</sup></u>     |                        |  |                                |                        |  | <u>0.0013N<sup>b</sup></u>     |                        |  |                                |                        |  |
|--------------------------------|------------------------|--|--------------------------------|------------------------|--|--------------------------------|------------------------|--|--------------------------------|------------------------|--|
| <u>Run 1</u>                   |                        |  | <u>Run 2</u>                   |                        |  | <u>Run 1</u>                   |                        |  | <u>Run 2</u>                   |                        |  |
| <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> |  | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> |  | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> |  | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> |  |
| 0                              | 0.735                  |  | 0                              | 0.754                  |  | 0                              | 0.734                  |  | 0                              | 0.734                  |  |
| 20.0                           | 0.698                  |  | 20.0                           | 0.717                  |  | 15.0                           | 0.699                  |  | 15.0**                         | 0.704                  |  |
| 40.0**                         | 0.670                  |  | 40.0**                         | 0.687                  |  | 30.0**                         | 0.672                  |  | 30.0**                         | 0.677                  |  |
| 60.0**                         | 0.646                  |  | 60.0**                         | 0.664                  |  | 45.0**                         | 0.648                  |  | 45.0**                         | 0.653                  |  |
| 80.0**                         | 0.624                  |  | 80.0**                         | 0.643                  |  | 60.0**                         | 0.627                  |  | 60.0**                         | 0.631                  |  |
| 100**                          | 0.605                  |  | 100**                          | 0.623                  |  | 80.0**                         | 0.604                  |  | 75.0**                         | 0.613                  |  |
| 125                            | 0.587                  |  | 125                            | 0.605                  |  | 100                            | 0.582                  |  | 90.0                           | 0.597                  |  |
| 150                            | 0.570                  |  | 150                            | 0.587                  |  | 125                            | 0.561                  |  | 110                            | 0.577                  |  |
| 200                            | 0.542                  |  | 175                            | 0.573                  |  | 150                            | 0.545                  |  | 135                            | 0.557                  |  |
| 250                            | 0.523                  |  | 200                            | 0.560                  |  | 175                            | 0.530                  |  | 160                            | 0.539                  |  |
| 300                            | 0.509                  |  | 250                            | 0.540                  |  | 200                            | 0.518                  |  | 185                            | 0.528                  |  |
| 350                            | 0.495                  |  | 300                            | 0.525                  |  | 250                            | 0.498                  |  | 225                            | 0.509                  |  |
| 400                            | 0.485                  |  | 350                            | 0.514                  |  | 300                            | 0.483                  |  | 275                            | 0.491                  |  |
| 500                            | 0.470                  |  | 400                            | 0.504                  |  | 350                            | 0.474                  |  | 325                            | 0.479                  |  |
| 600                            | 0.460                  |  | 500                            | 0.489                  |  | 400                            | 0.465                  |  | 375                            | 0.469                  |  |
| 800                            | 0.448                  |  | 600                            | 0.481                  |  | 450                            | 0.459                  |  | 425                            | 0.461                  |  |
| 1000                           | 0.439                  |  | 700                            | 0.473                  |  | 550                            | 0.450                  |  | 475                            | 0.457                  |  |
| 1200                           | 0.435                  |  | 900                            | 0.463                  |  | A <sub>∞</sub> <sup>e</sup>    | 0.418                  |  | 575                            | 0.448                  |  |
| A <sub>∞</sub> <sup>e</sup>    | 0.395                  |  | 1100                           | 0.458                  |  |                                |                        |  | 775                            | 0.435                  |  |
|                                |                        |  | A <sub>∞</sub> <sup>e</sup>    | 0.409                  |  |                                |                        |  | 1075                           | 0.427                  |  |
|                                |                        |  |                                |                        |  |                                |                        |  | A <sub>∞</sub> <sup>e</sup>    | 0.399                  |  |

Table 9 (Continued)

0.00063N<sup>b</sup>

| <u>Run 1</u>                   |                        | <u>Run 2</u>                   |                        |
|--------------------------------|------------------------|--------------------------------|------------------------|
| <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> |
| 0                              | 0.718                  | 0                              | 0.733                  |
| 10.0                           | 0.692                  | 20.0                           | 0.687                  |
| 25.0                           | 0.660**                | 40.0**                         | 0.649                  |
| 40.0                           | 0.632**                | 60.0**                         | 0.616                  |
| 55.0                           | 0.608                  | 80.0                           | 0.590                  |
| 70.0                           | 0.587                  | 100                            | 0.569                  |
| 85.0                           | 0.569                  | 125                            | 0.547                  |
| 100                            | 0.554                  | 150                            | 0.528                  |
| 125                            | 0.533                  | 175                            | 0.513                  |
| 150                            | 0.517                  | 200                            | 0.502                  |
| 175                            | 0.503                  | 250                            | 0.485                  |
| 200                            | 0.492                  | 300                            | 0.472                  |
| 250                            | 0.475                  | 350                            | 0.464                  |
| 300                            | 0.464                  | 400                            | 0.458                  |
| 350                            | 0.455                  | 500                            | 0.447                  |
| 400                            | 0.448                  | 600                            | 0.442                  |
| 500                            | 0.439                  | A <sub>∞</sub> <sup>e</sup>    | 0.416                  |
| 600                            | 0.434                  |                                |                        |
| 800                            | 0.426                  |                                |                        |
| 1000                           | 0.426                  |                                |                        |
| A <sub>∞</sub> <sup>e</sup>    | 0.393                  |                                |                        |

Table 9 (Continued)

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<sup>a</sup>T = 25.0 ± 0.1°C, pH = 5.90.

<sup>b</sup>Initial total concentration of supporting electrolyte.

<sup>c</sup>Total volume of solution of calf thymus DNA added (μl). Titrant contained 8.246 × 10<sup>-9</sup> moles of DNA phosphate per microliter.

<sup>d</sup>Total absorbance of sample at 365 nm, 4.00 cm pathlength. Data points marked (\*\*) used to compute K<sub>s</sub>.

<sup>e</sup>Calculated absorbance of solution if all 3-aminoacridinium cation is bound. Based on molar absorptivity of bound drug of 8494 M<sup>-1</sup>cm<sup>-1</sup>.

Table 10. Absorptiometric titration of 3-aminoacridinium cation with DNA, calcium salt. Calcium acetate as supporting electrolyte <sup>a</sup>

| <u>0.025N<sup>b</sup></u>   |                  |                             |                  | <u>0.010N<sup>b</sup></u>   |                  |                             |                  | <u>0.0050N<sup>b</sup></u>  |                  |                             |                  |
|-----------------------------|------------------|-----------------------------|------------------|-----------------------------|------------------|-----------------------------|------------------|-----------------------------|------------------|-----------------------------|------------------|
| <u>Run 1</u>                |                  | <u>Run 1</u>                |                  | <u>Run 1</u>                |                  | <u>Run 1</u>                |                  | <u>Run 1</u>                |                  | <u>Run 2</u>                |                  |
| Vol <sup>c</sup><br>DNA     | Abs <sup>d</sup> | Vol <sup>c</sup><br>DNA     | Abs <sup>d</sup> | Vol <sup>c</sup><br>DNA     | Abs <sup>d</sup> | Vol <sup>c</sup><br>DNA     | Abs <sup>d</sup> | Vol <sup>c</sup><br>DNA     | Abs <sup>d</sup> | Vol <sup>c</sup><br>DNA     | Abs <sup>d</sup> |
| 0                           | 0.694            | 0                           | 0.717            | 0                           | 0.735            | 0                           | 0.735            | 0                           | 0.704            | 0                           | 0.704            |
| 30.0                        | 0.669            | 20.0                        | 0.696            | 20.0                        | 0.696            | 10.0                        | 0.723            | 20.0                        | 0.673            | 20.0                        | 0.673            |
| 60.0**                      | 0.652            | 40.0**                      | 0.679            | 40.0**                      | 0.679            | 20.0                        | 0.713            | 40.0**                      | 0.651            | 40.0**                      | 0.651            |
| 90.0**                      | 0.637            | 60.0**                      | 0.660            | 60.0**                      | 0.660            | 50.0**                      | 0.681            | 60.0**                      | 0.634            | 60.0**                      | 0.634            |
| 140**                       | 0.614            | 80.0**                      | 0.647            | 80.0**                      | 0.647            | 80.0**                      | 0.652            | 80.0**                      | 0.616            | 80.0**                      | 0.616            |
| 190**                       | 0.595            | 110**                       | 0.627            | 110**                       | 0.627            | 110**                       | 0.629            | 110**                       | 0.595            | 110**                       | 0.595            |
| 240**                       | 0.578            | 140                         | 0.608            | 140                         | 0.608            | 140**                       | 0.610            | 140                         | 0.575            | 140                         | 0.575            |
| 290                         | 0.564            | 170                         | 0.593            | 170                         | 0.593            | 170**                       | 0.594            | 180                         | 0.555            | 180                         | 0.555            |
| 365                         | 0.546            | 210                         | 0.576            | 210                         | 0.576            | 200                         | 0.577            | 220                         | 0.537            | 220                         | 0.537            |
| 450                         | 0.527            | 250                         | 0.559            | 250                         | 0.559            | 250                         | 0.557            | 270                         | 0.519            | 270                         | 0.519            |
| 550                         | 0.512            | 300                         | 0.543            | 300                         | 0.543            | 300                         | 0.542            | 320                         | 0.506            | 320                         | 0.506            |
| 650                         | 0.498            | 350                         | 0.530            | 350                         | 0.530            | 350                         | 0.524            | 400                         | 0.488            | 400                         | 0.488            |
| 800                         | 0.482            | 400                         | 0.520            | 400                         | 0.520            | 400                         | 0.515            | 500                         | 0.473            | 500                         | 0.473            |
| 950                         | 0.470            | 500                         | 0.501            | 500                         | 0.501            | 500                         | 0.496            | 600                         | 0.460            | 600                         | 0.460            |
| 1250                        | 0.455            | 600                         | 0.485            | 600                         | 0.485            | 600                         | 0.483            | 800                         | 0.446            | 800                         | 0.446            |
| 1450                        | 0.449            | 700                         | 0.478            | 700                         | 0.478            | 700                         | 0.472            | 1100                        | 0.432            | 1100                        | 0.432            |
| A <sub>∞</sub> <sup>e</sup> | 0.365            | 900                         | 0.460            | 900                         | 0.460            | 900                         | 0.459            | A <sub>∞</sub> <sup>e</sup> | 0.381            | A <sub>∞</sub> <sup>e</sup> | 0.381            |
|                             |                  | 1100                        | 0.450            | 1100                        | 0.450            | 1200                        | 0.447            |                             |                  |                             |                  |
|                             |                  | 1475                        | 0.436            | 1475                        | 0.436            | 1600                        | 0.437            |                             |                  |                             |                  |
|                             |                  | A <sub>∞</sub> <sup>e</sup> | 0.376            | A <sub>∞</sub> <sup>e</sup> | 0.376            | A <sub>∞</sub> <sup>e</sup> | 0.381            |                             |                  |                             |                  |

Table 10 (Continued)

| <u>0.0025N<sup>b</sup></u>     |                        |                                |                        | <u>0.0013N<sup>b</sup></u>     |                        |                                |                        |
|--------------------------------|------------------------|--------------------------------|------------------------|--------------------------------|------------------------|--------------------------------|------------------------|
| <u>Run 1</u>                   |                        | <u>Run 2</u>                   |                        | <u>Run 1</u>                   |                        | <u>Run 2</u>                   |                        |
| <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> |
| 0                              | 0.728                  | 0                              | 0.712                  | 0                              | 0.708                  | 0                              | 0.729                  |
| 20.0                           | 0.695                  | 20.0                           | 0.682                  | 15.0                           | 0.665                  | 20.0                           | 0.698                  |
| 40.0**                         | 0.671                  | 40.0**                         | 0.657                  | 30.0**                         | 0.646                  | 40.0**                         | 0.672                  |
| 65.0**                         | 0.643                  | 60.0**                         | 0.634                  | 45.0**                         | 0.630                  | 60.0**                         | 0.648                  |
| 90.0**                         | 0.618                  | 80.0**                         | 0.614                  | 60.0**                         | 0.609                  | 80.0**                         | 0.630                  |
| 115**                          | 0.598                  | 100**                          | 0.598                  | 80.0**                         | 0.600                  | 100**                          | 0.612                  |
| 140**                          | 0.580                  | 130**                          | 0.577                  | 100                            | 0.581                  | 125**                          | 0.592                  |
| 175                            | 0.559                  | 160                            | 0.556                  | 125                            | 0.565                  | 150                            | 0.577                  |
| 210                            | 0.541                  | 190                            | 0.541                  | 150                            | 0.549                  | 175                            | 0.563                  |
| 260                            | 0.521                  | 240                            | 0.520                  | 200                            | 0.528                  | 200                            | 0.550                  |
| 310                            | 0.507                  | 290                            | 0.503                  | 500                            | 0.459                  | 250                            | 0.529                  |
| 385                            | 0.491                  | 340                            | 0.490                  | 600                            | 0.449                  | 300                            | 0.513                  |
| 460                            | 0.478                  | 440                            | 0.471                  | 800                            | 0.436                  | 350                            | 0.499                  |
| 560                            | 0.464                  | 540                            | 0.460                  | 1000                           | 0.429                  | 400                            | 0.489                  |
| 760                            | 0.458                  | 690                            | 0.449                  | 1330                           | 0.420                  | 500                            | 0.476                  |
| 860                            | 0.447                  | 890                            | 0.439                  | A <sub>∞</sub> <sup>e</sup>    | 0.376                  | 600                            | 0.465                  |
| 1160                           | 0.437                  | 1190                           | 0.429                  |                                |                        | 700                            | 0.456                  |
| 1360                           | 0.431                  | 1390                           | 0.427                  |                                |                        | 900                            | 0.444                  |
| A <sub>∞</sub> <sup>e</sup>    | 0.385                  | A <sub>∞</sub> <sup>e</sup>    | 0.376                  |                                |                        | 1200                           | 0.437                  |
|                                |                        |                                |                        |                                |                        | A <sub>∞</sub> <sup>e</sup>    | 0.391                  |



Table 10 (Continued)

| <u>0.00063N<sup>b</sup></u>    |                        |                                |                        | <u>0.000010N<sup>b</sup></u>   |                        |                                |                        |
|--------------------------------|------------------------|--------------------------------|------------------------|--------------------------------|------------------------|--------------------------------|------------------------|
| <u>Run 1</u>                   |                        | <u>Run 2</u>                   |                        | <u>Run 1</u>                   |                        | <u>Run 2</u>                   |                        |
| <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> |
| 0                              | 0.733                  | 0                              | 0.740                  | 0                              | 0.667                  | 0                              | 0.667                  |
| 10.0                           | 0.714                  | 15.0                           | 0.709                  | 10.0                           | 0.632                  | 10.0                           | 0.632                  |
| 20.0                           | 0.697                  | 30.0**                         | 0.684                  | 20.0**                         | 0.610                  | 20.0**                         | 0.610                  |
| 35.0**                         | 0.675                  | 45.0**                         | 0.663                  | 30.0**                         | 0.587                  | 30.0**                         | 0.587                  |
| 50.0**                         | 0.653                  | 60.0**                         | 0.643                  | 40.0**                         | 0.566                  | 40.0**                         | 0.566                  |
| 65.0**                         | 0.634                  | 80.0**                         | 0.612                  | 55.0**                         | 0.539                  | 55.0**                         | 0.539                  |
| 85.0**                         | 0.612                  | 100**                          | 0.600                  | 70.0                           | 0.520                  | 70.0                           | 0.520                  |
| 105**                          | 0.593                  | 125                            | 0.580                  | 85.0                           | 0.500                  | 85.0                           | 0.500                  |
| 125                            | 0.578                  | 150                            | 0.562                  | 105                            | 0.483                  | 105                            | 0.483                  |
| 150                            | 0.559                  | 175                            | 0.547                  | 125                            | 0.469                  | 125                            | 0.469                  |
| 175                            | 0.545                  | 225                            | 0.522                  | 145                            | 0.458                  | 145                            | 0.458                  |
| 475                            | 0.464                  | 275                            | 0.503                  | 170                            | 0.445                  | 170                            | 0.445                  |
| 575                            | 0.453                  | 325                            | 0.491                  | 220                            | 0.431                  | 220                            | 0.431                  |
| 675                            | 0.447                  | 425                            | 0.471                  | 270                            | 0.419                  | 270                            | 0.419                  |
| 975                            | 0.435                  | 525                            | 0.461                  | 370                            | 0.409                  | 370                            | 0.409                  |
| 1375                           | 0.428                  | 725                            | 0.448                  | 590                            | 0.398                  | 590                            | 0.398                  |
| A <sub>∞</sub> <sup>e</sup>    | 0.388                  | 925                            | 0.440                  | A <sub>∞</sub> <sup>e</sup>    | 0.379                  | A <sub>∞</sub> <sup>e</sup>    | 0.379                  |
|                                |                        | A <sub>∞</sub> <sup>e</sup>    | 0.406                  |                                |                        |                                |                        |

<sup>a</sup>T = 25.0 ± 0.1°C, pH = 5.90.

Table 10 (Continued)

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|                                                                                                                                                                               |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <sup>b</sup> Initial total concentration of supporting electrolyte.                                                                                                           |
| <sup>c</sup> Total volume of solution of calf thymus DNA added ( $\mu$ l). Titrant contained $7.935 \times 10^{-9}$ moles of DNA phosphate per microliter.                    |
| <sup>d</sup> Total absorbance of sample at 365 nm, 4.00 cm pathlength. Data points marked (**) used to compute $K_s$ .                                                        |
| <sup>e</sup> Calculated absorbance of solution if all 3-aminoacridinium cation is bound. Based on molar absorptivity of bound drug of $8494 \text{ M}^{-1} \text{ cm}^{-1}$ . |

Table 11. Absorptiometric titration of 3-aminoacridinium cation with DNA, strontium salt. Strontium acetate as supporting electrolyte a

| <u>0.025N<sup>b</sup></u>      |                        | <u>0.010N<sup>b</sup></u>      |                                |
|--------------------------------|------------------------|--------------------------------|--------------------------------|
| <u>Run 1</u>                   |                        | <u>Run 1</u>                   |                                |
| <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u>         |
| 0                              | 0.668                  | 0                              | 0.678                          |
| 25.0                           | 0.651                  | 20.0                           | 0.659                          |
| 50.0                           | 0.636                  | 40.0                           | 0.640                          |
| 75.0                           | 0.620                  | 60.0**                         | 0.623                          |
| 100**                          | 0.604                  | 80.0**                         | 0.608                          |
| 150**                          | 0.583                  | 100**                          | 0.593                          |
| 200**                          | 0.562                  | 125**                          | 0.577                          |
| 250**                          | 0.547                  | 150**                          | 0.564                          |
| 300                            | 0.532                  | 200                            | 0.542                          |
| 400                            | 0.509                  | 250                            | 0.524                          |
| 500                            | 0.494                  | 300                            | 0.508                          |
| 600                            | 0.481                  | 350                            | 0.497                          |
| 800                            | 0.463                  | 400                            | 0.485                          |
| 1000                           | 0.450                  | 500                            | 0.469                          |
| 1300                           | 0.440                  | 600                            | 0.457                          |
| 1525                           | 0.433                  | 800                            | 0.444                          |
| A <sub>∞</sub> <sup>e</sup>    | 0.349                  | 1100                           | 0.430                          |
|                                |                        | A <sub>∞</sub> <sup>e</sup>    | 0.367                          |
|                                |                        |                                |                                |
|                                |                        | <u>Run 2</u>                   | <u>Run 2</u>                   |
|                                |                        | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Vol<sup>c</sup><br/>DNA</u> |
|                                |                        | <u>Abs<sup>d</sup></u>         | <u>Abs<sup>d</sup></u>         |
|                                |                        | 0                              | 0                              |
|                                |                        | 25.0                           | 20.0                           |
|                                |                        | 50.0                           | 40.0                           |
|                                |                        | 75.0                           | 60.0**                         |
|                                |                        | 100**                          | 80.0**                         |
|                                |                        | 150**                          | 100**                          |
|                                |                        | 200**                          | 125**                          |
|                                |                        | 250**                          | 150**                          |
|                                |                        | 300                            | 200                            |
|                                |                        | 400                            | 250                            |
|                                |                        | 500                            | 300                            |
|                                |                        | 600                            | 350                            |
|                                |                        | 700                            | 400                            |
|                                |                        | 900                            | 500                            |
|                                |                        | 1100                           | 600                            |
|                                |                        | 1400                           | 800                            |
|                                |                        | 1725                           | 1000                           |
|                                |                        | A <sub>∞</sub> <sup>e</sup>    | A <sub>∞</sub> <sup>e</sup>    |
|                                |                        |                                |                                |
|                                |                        |                                | 0.677                          |
|                                |                        |                                | 0.654                          |
|                                |                        |                                | 0.636                          |
|                                |                        |                                | 0.619                          |
|                                |                        |                                | 0.607                          |
|                                |                        |                                | 0.594                          |
|                                |                        |                                | 0.580                          |
|                                |                        |                                | 0.564                          |
|                                |                        |                                | 0.540                          |
|                                |                        |                                | 0.523                          |
|                                |                        |                                | 0.507                          |
|                                |                        |                                | 0.494                          |
|                                |                        |                                | 0.483                          |
|                                |                        |                                | 0.468                          |
|                                |                        |                                | 0.457                          |
|                                |                        |                                | 0.442                          |
|                                |                        |                                | 0.430                          |
|                                |                        |                                | 0.370                          |

Table 11 (Continued)

0.0050N<sup>b</sup>

| Run 1                       |                  | Run 2                       |                  | Run 3                       |                  |
|-----------------------------|------------------|-----------------------------|------------------|-----------------------------|------------------|
| Vol <sup>c</sup><br>DNA     | Abs <sup>d</sup> | Vol <sup>c</sup><br>DNA     | Abs <sup>d</sup> | Vol <sup>c</sup><br>DNA     | Abs <sup>d</sup> |
| 0                           | 0.704            | 0                           | 0.648            | 0                           | 0.633            |
| 15.0                        | 0.657            | 20.0                        | 0.629            | 20.0                        | 0.604            |
| 30.0**                      | 0.639            | 40.0**                      | 0.602            | 40.0**                      | 0.583            |
| 50.0**                      | 0.617            | 60.0**                      | 0.583            | 60.0**                      | 0.562            |
| 75.0**                      | 0.597            | 80.0**                      | 0.567            | 80.0**                      | 0.546            |
| 100**                       | 0.578            | 100**                       | 0.552            | 100**                       | 0.529            |
| 125                         | 0.559            | 125**                       | 0.536            | 125**                       | 0.514            |
| 150                         | 0.544            | 150                         | 0.520            | 150                         | 0.497            |
| 175                         | 0.529            | 175                         | 0.509            | 175                         | 0.488            |
| 200                         | 0.518            | 200                         | 0.497            | 200                         | 0.477            |
| 250                         | 0.497            | 250                         | 0.479            | 250                         | 0.460            |
| 300                         | 0.481            | 300                         | 0.464            | 300                         | 0.447            |
| 350                         | 0.470            | 350                         | 0.453            | 350                         | 0.436            |
| 400                         | 0.461            | 400                         | 0.445            | 400                         | 0.429            |
| 500                         | 0.449            | 500                         | 0.433            | 500                         | 0.415            |
| 700                         | 0.432            | 600                         | 0.425            | 700                         | 0.400            |
| 900                         | 0.423            | 700                         | 0.419            | 900                         | 0.395            |
| 1200                        | 0.417            | 1000                        | 0.408            | A <sup>e</sup> <sub>∞</sub> | 0.349            |
| A <sup>e</sup> <sub>∞</sub> | 0.380            | A <sup>e</sup> <sub>∞</sub> | 0.354            |                             |                  |

Table 11 (Continued)

| <u>0.0025N<sup>b</sup></u>           |                        |                                      |                        | <u>0.0013N<sup>b</sup></u>           |                        |                                      |                        |
|--------------------------------------|------------------------|--------------------------------------|------------------------|--------------------------------------|------------------------|--------------------------------------|------------------------|
| <u>Run 1</u>                         |                        | <u>Run 2</u>                         |                        | <u>Run 1</u>                         |                        | <u>Run 2</u>                         |                        |
| <u>Vol<sub>C</sub></u><br><u>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sub>C</sub></u><br><u>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sub>C</sub></u><br><u>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sub>C</sub></u><br><u>DNA</u> | <u>Abs<sup>d</sup></u> |
| 0                                    | 0.660                  | 0                                    | 0.632                  | 0                                    | 0.701                  | 0                                    | 0.577                  |
| 20.0                                 | 0.632                  | 20.0                                 | 0.605                  | 20.0                                 | 0.662                  | 20.0                                 | 0.539                  |
| 40.0**                               | 0.607                  | 40.0**                               | 0.579                  | 40.0**                               | 0.629                  | 30.0**                               | 0.523                  |
| 60.0**                               | 0.583                  | 50.0**                               | 0.567                  | 60.0**                               | 0.600                  | 40.0**                               | 0.508                  |
| 80.0**                               | 0.551                  | 60.0**                               | 0.554                  | 80.0**                               | 0.578                  | 50.0**                               | 0.498                  |
| 100*                                 | 0.545                  | 80.0**                               | 0.532                  | 100                                  | 0.557                  | 60.0**                               | 0.487                  |
| 125                                  | 0.526                  | 100                                  | 0.517                  | 125                                  | 0.536                  | 80.0                                 | 0.467                  |
| 150                                  | 0.508                  | 125                                  | 0.494                  | 150                                  | 0.519                  | 100                                  | 0.450                  |
| 175                                  | 0.495                  | 150                                  | 0.479                  | 175                                  | 0.504                  | 125                                  | 0.433                  |
| 200                                  | 0.484                  | 175                                  | 0.465                  | 200                                  | 0.494                  | 150                                  | 0.420                  |
| 250                                  | 0.467                  | 200                                  | 0.453                  | 250                                  | 0.479                  | 175                                  | 0.411                  |
| 300                                  | 0.454                  | 250                                  | 0.438                  | 300                                  | 0.468                  | 200                                  | 0.402                  |
| 400                                  | 0.437                  | 300                                  | 0.424                  | 400                                  | 0.452                  | 250                                  | 0.390                  |
| 500                                  | 0.424                  | 350                                  | 0.417                  | 500                                  | 0.443                  | 300                                  | 0.380                  |
| 700                                  | 0.414                  | 400                                  | 0.413                  | 800                                  | 0.432                  | 350                                  | 0.376                  |
| 1000                                 | 0.407                  | 500                                  | 0.400                  | A <sub>∞</sub> <sup>e</sup>          | 0.390                  | 400                                  | 0.373                  |
| A <sub>∞</sub> <sup>e</sup>          | 0.361                  | 700                                  | 0.391                  |                                      |                        | 500                                  | 0.368                  |
|                                      |                        | 900                                  | 0.387                  |                                      |                        | A <sub>∞</sub> <sup>e</sup>          | 0.331                  |
|                                      |                        | A <sub>∞</sub> <sup>e</sup>          | 0.349                  |                                      |                        |                                      |                        |

Table 11 (Continued)

- 
- <sup>a</sup> $T = 25.0 \pm 0.1^{\circ}\text{C}$ ,  $\text{pH} = 5.90$ .
- <sup>b</sup>Initial total concentration of supporting electrolyte.
- <sup>c</sup>Total volume of solution of calf thymus DNA added ( $\mu\text{l}$ ). Titrant contained  $7.906 \times 10^{-9}$  moles of DNA phosphate per microliter.
- <sup>d</sup>Total absorbance of sample at 365 nm, 4.00 cm pathlength. Data points marked (\*\*) used to compute  $K_s$ .
- <sup>e</sup>Calculated absorbance of solution if all 3-aminoacridinium cation is bound. Based on molar absorptivity of bound drug of  $8494\text{M}^{-1}\text{cm}^{-1}$ .

Table 12. Absorptiometric titration of 3-aminoacridinium cation with DNA, barium salt.  
Barium acetate as supporting electrolyte <sup>a</sup>

| <u>0.010N<sup>b</sup></u>      |                        |                                |                        |
|--------------------------------|------------------------|--------------------------------|------------------------|
| <u>0.0050N<sup>b</sup></u>     |                        |                                |                        |
| <u>Run 1</u>                   |                        | <u>Run 2</u>                   |                        |
| <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> |
| 0                              | 0.734                  | 0                              | 0.739                  |
| 20.0                           | 0.707                  | 20.0                           | 0.713                  |
| 40.0                           | 0.683                  | 40.0                           | 0.688                  |
| 60.0**                         | 0.664                  | 60.0**                         | 0.669                  |
| 80.0**                         | 0.647                  | 80.0**                         | 0.650                  |
| 100**                          | 0.630                  | 100**                          | 0.636                  |
| 125**                          | 0.613                  | 125**                          | 0.617                  |
| 150**                          | 0.596                  | 150**                          | 0.605                  |
| 200**                          | 0.568                  | 175                            | 0.592                  |
| 250                            | 0.548                  | 200                            | 0.582                  |
| 300                            | 0.531                  | 250                            | 0.560                  |
| 350                            | 0.519                  | 300                            | 0.539                  |
| 400                            | 0.508                  | 350                            | 0.528                  |
| 450                            | 0.497                  | 400                            | 0.517                  |
| 550                            | 0.484                  | 500                            | 0.499                  |
| 650                            | 0.472                  | 600                            | 0.489                  |
| 750                            | 0.465                  | 700                            | 0.479                  |
| 950                            | 0.454                  | 900                            | 0.470                  |
| 1150                           | 0.449                  | 1100                           | 0.465                  |
| 1350                           | 0.444                  | 1300                           | 0.464                  |
| 1650                           | 0.443                  | 1700                           | 0.463                  |
| A <sub>∞</sub> <sup>e</sup>    | 0.379                  | A <sub>∞</sub> <sup>e</sup>    | 0.380                  |
| <u>Run 1</u>                   |                        | <u>Run 2</u>                   |                        |
| <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> |
| 0                              | 0.740                  | 0                              | 0.734                  |
| 15.0                           | 0.718                  | 20.0                           | 0.701                  |
| 30.0                           | 0.695                  | 40.0**                         | 0.673                  |
| 50.0**                         | 0.672                  | 60.0**                         | 0.649                  |
| 70.0**                         | 0.646                  | 80.0**                         | 0.627                  |
| 90.0**                         | 0.624                  | 100**                          | 0.608                  |
| 110**                          | 0.607                  | 125                            | 0.586                  |
| 130                            | 0.591                  | 150                            | 0.570                  |
| 150                            | 0.579                  | 175                            | 0.555                  |
| 175                            | 0.564                  | 200                            | 0.542                  |
| 200                            | 0.551                  | 250                            | 0.520                  |
| 250                            | 0.531                  | 300                            | 0.505                  |
| 300                            | 0.513                  | 350                            | 0.492                  |
| 350                            | 0.501                  | 400                            | 0.480                  |
| 400                            | 0.493                  | 500                            | 0.469                  |
| 500                            | 0.477                  | 600                            | 0.460                  |
| 600                            | 0.466                  | 700                            | 0.453                  |
| 700                            | 0.459                  | 900                            | 0.447                  |
| 800                            | 0.455                  | 1200                           | 0.441                  |
| 1000                           | 0.452                  | A <sub>∞</sub> <sup>e</sup>    | 0.394                  |
| 1300                           | 0.448                  |                                |                        |
| A <sub>∞</sub> <sup>e</sup>    | 0.394                  |                                |                        |

Table 12 (Continued)

| <u>0.0025N<sup>b</sup></u>           |                        |                                      |                        | <u>0.0013N<sup>b</sup></u>           |                        |                                      |                        |
|--------------------------------------|------------------------|--------------------------------------|------------------------|--------------------------------------|------------------------|--------------------------------------|------------------------|
| <u>Run 1</u>                         |                        | <u>Run 2</u>                         |                        | <u>Run 1</u>                         |                        | <u>Run 2</u>                         |                        |
| <u>Vol<sub>C</sub></u><br><u>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sub>C</sub></u><br><u>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sub>C</sub></u><br><u>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sub>C</sub></u><br><u>DNA</u> | <u>Abs<sup>d</sup></u> |
| 0                                    | 0.718                  | 0                                    | 0.733                  | 0                                    | 0.729                  | 0                                    | 0.735                  |
| 15.0                                 | 0.688                  | 20.0                                 | 0.698                  | 15.0                                 | 0.693                  | 10.0                                 | 0.717                  |
| 30.0**                               | 0.663                  | 40.0**                               | 0.659                  | 30.0**                               | 0.661                  | 20.0**                               | 0.692                  |
| 45.0**                               | 0.641                  | 60.0**                               | 0.632                  | 45.0**                               | 0.633                  | 30.0**                               | 0.673                  |
| 60.0**                               | 0.620                  | 80.0**                               | 0.609                  | 60.0**                               | 0.608                  | 40.0**                               | 0.656                  |
| 80.0**                               | 0.599                  | 100**                                | 0.588                  | 75.0                                 | 0.587                  | 60.0**                               | 0.623                  |
| 100**                                | 0.578                  | 125                                  | 0.567                  | 90.0                                 | 0.569                  | 80.0**                               | 0.597                  |
| 120                                  | 0.562                  | 150                                  | 0.549                  | 110                                  | 0.550                  | 100                                  | 0.576                  |
| 140                                  | 0.547                  | 175                                  | 0.534                  | 130                                  | 0.535                  | 125                                  | 0.552                  |
| 165                                  | 0.532                  | 200                                  | 0.521                  | 155                                  | 0.517                  | 150                                  | 0.535                  |
| 190                                  | 0.517                  | 225                                  | 0.512                  | 180                                  | 0.504                  | 175                                  | 0.520                  |
| 225                                  | 0.502                  | 275                                  | 0.494                  | 205                                  | 0.494                  | 200                                  | 0.509                  |
| 275                                  | 0.486                  | 325                                  | 0.483                  | 255                                  | 0.478                  | 250                                  | 0.491                  |
| 325                                  | 0.474                  | 375                                  | 0.473                  | 305                                  | 0.465                  | 300                                  | 0.479                  |
| 375                                  | 0.463                  | 425                                  | 0.465                  | 355                                  | 0.456                  | 350                                  | 0.471                  |
| 425                                  | 0.456                  | 525                                  | 0.455                  | 455                                  | 0.446                  | 400                                  | 0.468                  |
| 500                                  | 0.449                  | 625                                  | 0.449                  | 555                                  | 0.442                  | 500                                  | 0.460                  |
| 600                                  | 0.441                  | 725                                  | 0.445                  | 855                                  | 0.437                  | 650                                  | 0.457                  |
| 750                                  | 0.435                  | 1025                                 | 0.438                  | A <sub>∞</sub> <sup>e</sup>          | 0.404                  | 950                                  | 0.450                  |
| 950                                  | 0.430                  | 1350                                 | 0.437                  |                                      |                        | 1150                                 | 0.450                  |
| 1250                                 | 0.429                  | A <sub>∞</sub> <sup>e</sup>          | 0.388                  |                                      |                        | A <sub>∞</sub> <sup>e</sup>          | 0.396                  |
| A <sub>∞</sub> <sup>e</sup>          | 0.384                  |                                      |                        |                                      |                        |                                      |                        |



Table 12 (Continued)

| <u>0.00063N<sup>b</sup></u>    |                        |                                |                        | <u>1.0 x 10<sup>-5</sup>N<sup>b</sup></u> |                        |                                |                        |
|--------------------------------|------------------------|--------------------------------|------------------------|-------------------------------------------|------------------------|--------------------------------|------------------------|
| <u>Run 1</u>                   |                        | <u>Run 2</u>                   |                        | <u>Run 1</u>                              |                        | <u>Run 2</u>                   |                        |
| <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> | <u>Vol<sup>c</sup><br/>DNA</u>            | <u>Abs<sup>d</sup></u> | <u>Vol<sup>c</sup><br/>DNA</u> | <u>Abs<sup>d</sup></u> |
| 0                              | 0.726                  | 0                              | 0.728                  | 0                                         | 0.680                  | 0                              | 0.698                  |
| 10.0                           | 0.692                  | 10.0                           | 0.701                  | 10.0**                                    | 0.644                  | 10.0                           | 0.674                  |
| 20.0**                         | 0.670                  | 20.0**                         | 0.676                  | 20.0**                                    | 0.610                  | 20.0**                         | 0.648                  |
| 30.0**                         | 0.650                  | 30.0**                         | 0.653                  | 30.0**                                    | 0.582                  | 30.0**                         | 0.626                  |
| 45.0**                         | 0.621                  | 40.0**                         | 0.633                  | 40.0**                                    | 0.558                  | 40.0**                         | 0.607                  |
| 60.0                           | 0.598                  | 50.0**                         | 0.617                  | 50.0                                      | 0.535                  | 55.0**                         | 0.581                  |
| 75.0                           | 0.576                  | 65.0                           | 0.591                  | 65.0                                      | 0.517                  | 70.0                           | 0.559                  |
| 90.0                           | 0.559                  | 80.0                           | 0.570                  | 80.0                                      | 0.491                  | 85.0                           | 0.539                  |
| 110                            | 0.538                  | 100                            | 0.549                  | 95.0                                      | 0.474                  | 105                            | 0.516                  |
| 130                            | 0.520                  | 120                            | 0.531                  | 115                                       | 0.450                  | 125                            | 0.499                  |
| 155                            | 0.503                  | 140                            | 0.516                  | 135                                       | 0.437                  | 145                            | 0.488                  |
| 180                            | 0.493                  | 160                            | 0.502                  | 160                                       | 0.425                  | 170                            | 0.475                  |
| 230                            | 0.476                  | 190                            | 0.488                  | 210                                       | 0.413                  | 195                            | 0.465                  |
| 280                            | 0.463                  | 220                            | 0.476                  | 260                                       | 0.404                  | 245                            | 0.450                  |
| 330                            | 0.455                  | 250                            | 0.467                  | 360                                       | 0.400                  | 295                            | 0.441                  |
| 430                            | 0.447                  | 300                            | 0.458                  | 520                                       | 0.400                  | 395                            | 0.429                  |
| 530                            | 0.442                  | 350                            | 0.451                  | A <sub>∞</sub> <sup>e</sup>               | 0.389                  | 595                            | 0.405                  |
| 730                            | 0.438                  | 450                            | 0.443                  |                                           |                        | A <sub>∞</sub> <sup>e</sup>    | 0.396                  |
| A <sub>∞</sub> <sup>e</sup>    | 0.407                  | 550                            | 0.440                  |                                           |                        |                                |                        |
|                                |                        | 850                            | 0.438                  |                                           |                        |                                |                        |
|                                |                        | A <sub>∞</sub> <sup>e</sup>    | 0.404                  |                                           |                        |                                |                        |

Table 12 (Continued)

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| <u>1.0 x 10<sup>-5</sup>N<sup>b</sup></u> |                        |
|-------------------------------------------|------------------------|
| <hr/>                                     |                        |
| <u>Run 3</u>                              |                        |
| <u>Vol<sup>c</sup><br/>DNA</u>            | <u>Abs<sup>d</sup></u> |
| 0                                         | 0.683                  |
| 10.0**                                    | 0.642                  |
| 20.0**                                    | 0.610                  |
| 30.0**                                    | 0.583                  |
| 40.0**                                    | 0.557                  |
| 50.0                                      | 0.539                  |
| 65.0                                      | 0.510                  |
| 80.0                                      | 0.489                  |
| 95.0                                      | 0.474                  |
| 115                                       | 0.457                  |
| 135                                       | 0.443                  |
| 160                                       | 0.434                  |
| 210                                       | 0.420                  |
| 260                                       | 0.417                  |
| 360                                       | 0.411                  |
| 560                                       | 0.409                  |
| A <sub>∞</sub> <sup>e</sup>               | 0.389                  |

---

<sup>a</sup>T = 25.0 ± 0.1°C, pH = 5.90.<sup>b</sup>Initial total concentration of supporting electrolyte.

Table 12 (Continued)

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|                                                                                                                                                                           |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <sup>c</sup> Total volume of solution of calf thymus DNA added ( $\mu$ l). Titrant contained $8.184 \times 10^{-9}$ moles of DNA phosphate per microliter.                |
| <sup>d</sup> Total absorbance of sample at 365 nm, 4.00 cm pathlength. Data points marked (**) used to compute $K_s$ .                                                    |
| <sup>e</sup> Calculated absorbance of solution if all 3-aminoacridinium cation is bound. Based on molar absorptivity of bound drug of $8494\text{M}^{-1}\text{cm}^{-1}$ . |

Table 13. Absorptiometric titration of 3-aminoacridinium cation with DNA, potassium salt, at 15°C.<sup>a</sup>

| Run 1                       |                  |  | Run 2                       |                  |  | Run 3                       |                  |  |
|-----------------------------|------------------|--|-----------------------------|------------------|--|-----------------------------|------------------|--|
| Vol <sup>b</sup><br>DNA     | Abs <sup>c</sup> |  | Vol <sup>b</sup><br>DNA     | Abs <sup>c</sup> |  | Vol <sup>b</sup><br>DNA     | Abs <sup>c</sup> |  |
| 0                           | 0.717            |  | 0                           | 0.653            |  | 0                           | 0.668            |  |
| 7.5                         | 0.689            |  | 7.5**                       | 0.622            |  | 7.5                         | 0.640            |  |
| 15.0**                      | 0.654            |  | 15.0**                      | 0.596            |  | 15.0**                      | 0.615            |  |
| 22.5**                      | 0.628            |  | 22.5**                      | 0.573            |  | 22.5**                      | 0.590            |  |
| 30.0**                      | 0.601            |  | 30.0**                      | 0.549            |  | 30.0**                      | 0.568            |  |
| 40.0**                      | 0.573            |  | 40.0                        | 0.519            |  | 40.0                        | 0.539            |  |
| 50.0                        | 0.544            |  | 50.0                        | 0.492            |  | 50.0                        | 0.511            |  |
| 60.0*                       | 0.520            |  | 60.0*                       | 0.470            |  | 60.0*                       | 0.489            |  |
| 70.0*                       | 0.500            |  | 70.0*                       | 0.451            |  | 70.0*                       | 0.468            |  |
| 80.0*                       | 0.482            |  | 80.0*                       | 0.437            |  | 80.0*                       | 0.452            |  |
| 95.0*                       | 0.463            |  | 95.0                        | 0.428            |  | 95.0*                       | 0.429            |  |
| 110                         | 0.450            |  | 110                         | 0.407            |  | 110                         | 0.414            |  |
| 130                         | 0.441            |  | 130                         | 0.398            |  | 130                         | 0.401            |  |
| 180                         | 0.431            |  | 230                         | 0.393            |  | 230                         | 0.395            |  |
| 280                         | 0.429            |  | A <sub>∞</sub> <sup>d</sup> | 0.               |  | A <sub>∞</sub> <sup>d</sup> | 0.               |  |
| A <sub>∞</sub> <sup>d</sup> | 0.               |  |                             |                  |  |                             |                  |  |

<sup>a</sup>Potassium dihydrogen phosphate as supporting electrolyte, 0.010M initial total concentration of supporting electrolyte: 0.010M, pH = 5.90, temperature, 15.0 ± 0.2°C.

<sup>b</sup>Total volume of solution of calf thymus DNA added (μl). Titrant contained 8.132 × 10<sup>-9</sup> moles of DNA phosphate per microliter.

Table 13 (Continued)

---

<sup>c</sup>Total absorbance of sample, 4.00 cm pathlength. Data marked (\*\*) used to compute  $K_s$ , those marked (\*) were used to compute  $K_1$ .

<sup>d</sup>Calculated absorbance of solution if all 3-aminoacridinium cation is bound. Based on molar absorptivity of bound species of  $8494\text{M}^{-1}\text{cm}^{-1}$ .

Table 14. Absorptiometric titration of 3-aminoacridinium cation with DNA, potassium salt, at 35°C<sup>a</sup>

| Run 1                          |                        | Run 2                          |                        |
|--------------------------------|------------------------|--------------------------------|------------------------|
| <u>Vol<sup>b</sup><br/>DNA</u> | <u>Abs<sup>c</sup></u> | <u>Vol<sup>b</sup><br/>DNA</u> | <u>Abs<sup>c</sup></u> |
| 0                              | 0.683                  | 0                              | 0.671                  |
| 7.5                            | 0.658                  | 7.5                            | 0.638                  |
| 15.0**                         | 0.636                  | 15.0**                         | 0.621                  |
| 22.5**                         | 0.617                  | 22.5**                         | 0.600                  |
| 30.0**                         | 0.593                  | 30.0**                         | 0.583                  |
| 40.0**                         | 0.570                  | 40.0                           | 0.561                  |
| 50.0                           | 0.547                  | 50.0                           | 0.540                  |
| 60.0                           | 0.528                  | 60.0                           | 0.521                  |
| 70.0                           | 0.509                  | 70.0                           | 0.502                  |
| 80.0                           | 0.495                  | 80.0*                          | 0.488                  |
| 95.0*                          | 0.477                  | 95.0*                          | 0.467                  |
| 110*                           | 0.459                  | 110*                           | 0.450                  |
| 130*                           | 0.443                  | 130*                           | 0.430                  |
| 155*                           | 0.432                  | 155*                           | 0.419                  |
| 205                            | 0.427                  | 205                            | 0.405                  |
| 305                            | 0.420                  | 305                            | 0.403                  |
| 405                            | 0.420                  | A <sub>∞</sub> <sup>d</sup>    | 0.390                  |
| A <sub>∞</sub> <sup>d</sup>    | 0.393                  |                                |                        |

<sup>a</sup>Potassium dihydrogen phosphate as supporting electrolyte, 0.010M initial total concentration of supporting electrolyte, pH 5.90, temperature 35.0 ± 0.2°C.

<sup>b</sup>Total volume of solution of calf thymus DNA added (μl). Titrant contained 8.132 × 10<sup>-9</sup> moles of DNA phosphate per microliter.

Table 14 (Continued)

---

<sup>c</sup>Total absorbance of sample, 4.00 cm pathlength. Data marked (\*\*) used to compute  $K_s$ , those marked (\*) were used to compute  $K_1$ .

<sup>d</sup>Calculated absorbance of solution if all 3-aminoacridinium cation is bound. Based on molar absorptivity of bound species of  $8494\text{M}^{-1}\text{cm}^{-1}$ .

Table 15 . Absorptiometric titration of 7-aminoquinolinium cation with DNA, potassium salt, at 15°C a

| Run 1                 |                  | Run 2                 |                  |
|-----------------------|------------------|-----------------------|------------------|
| $\text{Vol}_b$<br>DNA | Abs <sup>c</sup> | $\text{Vol}_b$<br>DNA | Abs <sup>c</sup> |
| 0                     | 0.341            | 0                     | 0.331            |
| 50.0**                | 0.323            | 25.0                  | 0.325            |
| 75.0**                | 0.314            | 50.0**                | 0.315            |
| 100**                 | 0.309            | 75.0**                | 0.312            |
| 150                   | 0.298            | 125                   | 0.299            |
| 200*                  | 0.289            | 175                   | 0.290            |
| 250*                  | 0.281            | 225*                  | 0.282            |
| 300*                  | 0.275            | 275*                  | 0.273            |
| 400                   | 0.264            | 375*                  | 0.263            |
| 500                   | 0.255            | 475                   | 0.252            |
| 700                   | 0.247            | 675                   | 0.241            |
| 1000                  | 0.233            | 875                   | 0.231            |
| $A_\infty^d$          | 0.240            | $A_\infty^d$          | 0.236            |

<sup>a</sup> Potassium dihydrogen phosphate as supporting electrolyte, 0.010M initial total concentration, pH = 5.90, temperature, 15.0 ± 0.2°C.

<sup>b</sup> Total volume of solution of calf thymus DNA added. Titrant contained  $8.163 \times 10^{-9}$  moles DNA phosphate per microliter.

<sup>c</sup> Total absorbance of sample, 4.00 cm pathlength. Data marked (\*\*) used to compute  $K_s$ , those marked (\*) were used to compute  $K_1$ .



Table 15 (Continued)

---

<sup>d</sup>Calculated absorbance of solution if all 7-aminoquinolinium originally in 1 solution were bound. Based on a molar absorptivity of  $5332\text{M}^{-1}\text{cm}^{-1}$ .

Table 16. Absorptiometric titration of 7-aminoquinolinium cation with DNA, potassium salt, at 25°C

| Run 1                             |                  | Run 2                             |                  |
|-----------------------------------|------------------|-----------------------------------|------------------|
| $\frac{\text{Vol}_b}{\text{DNA}}$ | Abs <sup>c</sup> | $\frac{\text{Vol}_b}{\text{DNA}}$ | Abs <sup>c</sup> |
| 0                                 | 0.362            | 0                                 | 0.360            |
| 50.0                              | 0.353            | 50.0                              | 0.352            |
| 75.0**                            | 0.344            | 75.0**                            | 0.346            |
| 100**                             | 0.338            | 100**                             | 0.337            |
| 150**                             | 0.327            | 150**                             | 0.324            |
| 200                               | 0.318            | 200                               | 0.316            |
| 250*                              | 0.311            | 250*                              | 0.303            |
| 300*                              | 0.302            | 300*                              | 0.297            |
| 400*                              | 0.294            | 400*                              | 0.285            |
| 500*                              | 0.286            | 500*                              | 0.287            |
| 700*                              | 0.271            | 600*                              | 0.278            |
| 1000                              | 0.259            | 900                               | 0.264            |
| 1400                              | 0.243            | 1200                              | 0.255            |
| A <sub>∞</sub> <sup>d</sup>       | 0.244            | 1680                              | 0.238            |
|                                   |                  | A <sub>∞</sub> <sup>d</sup>       | 0.235            |

<sup>a</sup> Potassium dihydrogen phosphate as supporting electrolyte, 0.010M initial total concentration, pH = 5.90, temperature, 25.0 ± 0.1°C.

<sup>b</sup> Total volume of solution of calf thymus DNA added (μl). Titrant contained 8.163 × 10<sup>-9</sup> moles of DNA phosphate per microliter.

Table 16(Continued)

---

<sup>c</sup> Total absorbance of sample, 4.00 cm pathlength. Data marked (\*\*) used to compute  $K_s$ , those marked (\*) were used to compute  $K_1$ .

<sup>d</sup> Calculated absorbance of solution if all 7-aminoquinolinium originally in solution were bound. Based on a molar absorptivity of  $5332\text{M}^{-1}\text{cm}^{-1}$ .

Table 17. Absorptiometric titration of 7-aminoquinolinium cation with DNA, potassium salt, at 35°C a

| Run 1                             |                  | Run 2                             |                  | Run 3                             |                  | Run 4                             |                  |
|-----------------------------------|------------------|-----------------------------------|------------------|-----------------------------------|------------------|-----------------------------------|------------------|
| $\frac{\text{Vol}_b}{\text{DNA}}$ | Abs <sup>c</sup> | $\frac{\text{Vol}_b}{\text{DNA}}$ | Abs <sup>c</sup> | $\frac{\text{Vol}_b}{\text{DNA}}$ | Abs <sup>c</sup> | $\frac{\text{Vol}_b}{\text{DNA}}$ | Abs <sup>c</sup> |
| 0                                 | 0.342            | 0                                 | 0.326            | 0                                 | 0.326            | 0                                 | 0.316            |
| 50.0                              | 0.329            | 50.0                              | 0.317            | 50.0                              | 0.317            | 50.0                              | 0.307            |
| 75.0**                            | 0.324            | 100**                             | 0.309            | 75.0**                            | 0.311            | 100                               | 0.297            |
| 100**                             | 0.321            | 150**                             | 0.300            | 100**                             | 0.308            | 150                               | 0.289            |
| 150**                             | 0.309            | 200**                             | 0.293            | 150**                             | 0.297            | 200                               | 0.279            |
| 200                               | 0.307            | 250                               | 0.285            | 200                               | 0.289            | 250                               | 0.273            |
| 250                               | 0.300            | 300                               | 0.280            | 250                               | 0.284            | 300                               | 0.269            |
| 300*                              | 0.293            | 400*                              | 0.268            | 300                               | 0.278            | 400                               | 0.261            |
| 400*                              | 0.282            | 600*                              | 0.261            | 400*                              | 0.271            | 600                               | 0.253            |
| 500*                              | 0.276            | 900*                              | 0.242            | 500*                              | 0.265            | 800                               | 0.243            |
| 700*                              | 0.259            | 1300                              | 0.229            | 700*                              | 0.251            | 1100                              | 0.229            |
| 900*                              | 0.251            | 1800                              | 0.215            | 1000*                             | 0.243            | 1380                              | 0.225            |
| 1200                              | 0.242            | 2400                              | 0.195            | 1400                              | 0.226            | 1880                              | 0.208            |
| 1650                              | 0.227            | 3000                              | 0.185            | 1900                              | 0.215            |                                   |                  |
| A <sub>∞</sub> <sup>d</sup>       | 0.224            | A <sub>∞</sub> <sup>d</sup>       | 0.159            | 2580                              | 0.196            |                                   |                  |
|                                   |                  |                                   |                  | A <sub>∞</sub> <sup>d</sup>       | 0.203            |                                   |                  |

<sup>a</sup>Potassium dihydrogen phosphate as supporting electrolyte, 0.010M initial total concentration, pH = 5.90, temperature, 35.0 ± 0.2°C.

<sup>b</sup>Total volume of solution of calf thymus DNA added (μl). Titrant contained 8.163 × 10<sup>-9</sup> moles of DNA phosphate per microliter.

Table 17 (Continued)

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<sup>c</sup>Total absorbance of sample, 4.00 cm pathlength. Data marked (\*\*) used to compute  $K_s$ , those marked (\*) were used to compute  $K_1$ .

<sup>d</sup>Calculated absorbance of solution if all 7-aminoquinolinium originally in solution were bound. Based on a molar absorptivity of bound species of  $5332\text{M}^{-1}\text{cm}^{-1}$ .

Table 18. Calculated values of [BH], [BHP], "K<sub>s</sub>", and related data for the reaction between 3-aminoacridinium cation and DNA in 0.15M CsH<sub>2</sub>PO<sub>4</sub><sup>a</sup>

| Vol<br>DNA<br>(μl) | Abs   | [BH]<br>M<br>x 10 <sup>5</sup> | [BHP]<br>M<br>x 10 <sup>5</sup> | "K <sub>I</sub> " <sup>b</sup><br>M <sup>-2</sup><br>x 10 <sup>-8</sup> | -Log ([P <sub>t</sub> ]/ <sup>2</sup> -2[BHP]) | Log ([BHP]/[BH]) | K <sub>s</sub><br>M <sup>-1</sup><br>x 10 <sup>-4</sup> | -Log ([P <sub>t</sub> ]/ <sup>3</sup> -[BHP]) |
|--------------------|-------|--------------------------------|---------------------------------|-------------------------------------------------------------------------|------------------------------------------------|------------------|---------------------------------------------------------|-----------------------------------------------|
| 0                  | 0.763 | 1.350                          | 0                               | -                                                                       | -                                              | -                | -                                                       | -                                             |
| 15.0               | 0.738 | 1.251                          | .097                            | 37.5                                                                    | 5.34                                           | -1.11            | 2.31                                                    | 5.47                                          |
| 30.0**             | 0.716 | 1.164                          | .183                            | 18.0                                                                    | 5.03                                           | - .804           | 2.30                                                    | 5.17                                          |
| 45.0**             | 0.692 | 1.069                          | .275                            | 13.3                                                                    | 4.86                                           | - .589           | 2.52                                                    | 4.99                                          |
| 60.0**             | 0.672 | .990                           | .353                            | 10.0                                                                    | 4.73                                           | - .448           | 2.59                                                    | 4.86                                          |
| 75.0**             | 0.654 | .919                           | .422                            | 8.05                                                                    | 4.62                                           | - .338           | 2.65                                                    | 4.76                                          |
| 90.0**             | 0.635 | .844                           | .494                            | 7.05                                                                    | 4.54                                           | - .232           | 2.81                                                    | 4.68                                          |
| 110**              | 0.613 | .757                           | .578                            | 6.00                                                                    | 4.45                                           | - .117           | 2.97                                                    | 4.59                                          |
| 130                | 0.596 | .691                           | .642                            | 5.07                                                                    | 4.37                                           | - .031           | 3.03                                                    | 4.51                                          |
| 150                | 0.582 | .635                           | .695                            | 4.33                                                                    | 4.30                                           | + .039           | 3.05                                                    | 4.45                                          |
| 170                | 0.570 | .588                           | .740                            | 3.77                                                                    | 4.24                                           | + .099           | 3.07                                                    | 4.39                                          |
| 195                | 0.552 | .518                           | .807                            | 3.48                                                                    | 4.17                                           | + .192           | 3.29                                                    | 4.33                                          |
| 225*               | 0.540 | .471                           | .850                            | 2.93                                                                    | 4.11                                           | + .257           | 3.27                                                    | 4.26                                          |
| 265*               | 0.521 | .397                           | .919                            | 2.64                                                                    | 4.03                                           | + .365           | 3.54                                                    | 4.18                                          |
| 315*               | 0.507 | .342                           | .967                            | 2.21                                                                    | 3.95                                           | + .452           | 3.60                                                    | 4.10                                          |
| 365*               | 0.497 | .303                           | 1.000                           | 1.87                                                                    | 3.88                                           | + .519           | 3.60                                                    | 4.04                                          |

| Vol<br>DNA<br>( $\mu$ l) | Abs   | [BH]<br>M<br>$\times 10^5$ | [BHP]<br>M<br>$\times 10^5$ | "K <sub>I</sub> " <sup>b</sup><br>M <sup>-2</sup><br>$\times 10^{-8}$ | $-\log([P_t]/_2 - 2[BHP])$ | $\log([BHP]/[BH])$ | K <sub>S</sub><br>M <sup>-1</sup><br>$\times 10^{-4}$ | $-\log([P_t]/_3 - [BHP])$ |
|--------------------------|-------|----------------------------|-----------------------------|-----------------------------------------------------------------------|----------------------------|--------------------|-------------------------------------------------------|---------------------------|
| 415*                     | 0.488 | .268                       | 1.029                       | 1.66                                                                  | 3.82                       | +.585              | 3.66                                                  | 3.98                      |
| 515*                     | 0.474 | .213                       | 1.071                       | 1.38                                                                  | 3.72                       | +.701              | 3.84                                                  | 3.88                      |
| 715*                     | 0.460 | .158                       | 1.102                       | .973                                                                  | 3.57                       | +.843              | 3.83                                                  | 3.74                      |
| 1015                     | 0.454 | .133                       | 1.093                       | .577                                                                  | 3.42                       | +.916              | 3.22                                                  | 3.59                      |

<sup>a</sup>Refer to Table 7 for reaction conditions.

<sup>b</sup>Not a valid association constant as the intercalative process was not seen under these conditions. Included for illustrative purposes only. See text.

Table 19. Calculated values of [BH], [BHP],  $K_S$ ,  $K_I$ , and related data for the reaction between 3-aminoacridinium cation and DNA in 0.0025M  $\text{CsH}_2\text{PO}_4^a$

| Vol<br>DNA<br>( $\mu\text{l}$ ) | Abs   | [BH]<br>M<br>$\times 10^5$ | [BHP]<br>M<br>$\times 10^5$ | $K_I$<br>$\text{M}^{-2}$<br>$\times 10^{-10}$ | $-\log([P_t]/^2 - 2[\text{BHP}])$ | $\log([\text{BHP}]/[\text{BH}])$ | $K_S$<br>$\text{M}^{-1}$<br>$\times 10^{-5}$ | $-\log([P_t]/_3 - [\text{BHP}])$ |
|---------------------------------|-------|----------------------------|-----------------------------|-----------------------------------------------|-----------------------------------|----------------------------------|----------------------------------------------|----------------------------------|
| 0                               | 0.752 | 1.331                      | 0                           | 793                                           | 6.99                              | -1.07                            | 2.04                                         | 6.38                             |
| 5.0                             | 0.728 | 1.227                      | .103                        | -                                             | -                                 | -                                | -                                            | -                                |
| 10.0                            | 0.701 | 1.110                      | .219                        | -                                             | -                                 | -                                | -                                            | -                                |
| 15.0**                          | 0.681 | 1.024                      | .305                        | 191                                           | 6.40                              | .526                             | 2.33                                         | 5.89                             |
| 20.0**                          | 0.666 | .959                       | .370                        | 23.9                                          | 5.90                              | .414                             | 1.85                                         | 5.68                             |
| 25.0**                          | 0.649 | .885                       | .442                        | 12.8                                          | 5.70                              | .301                             | 1.79                                         | 5.55                             |
| 30.0**                          | 0.631 | .808                       | .519                        | 9.58                                          | 5.59                              | .192                             | 1.86                                         | 5.46                             |
| 37.5                            | 0.605 | .695                       | .631                        | 6.99                                          | 5.44                              | .042                             | 2.01                                         | 5.35                             |
| 45.0                            | 0.581 | .592                       | .733                        | 5.42                                          | 5.32                              | .093                             | 2.20                                         | 5.25                             |
| 52.5*                           | 0.561 | .506                       | .818                        | 4.07                                          | 5.20                              | .209                             | 2.33                                         | 5.16                             |
| 60.0*                           | 0.539 | .411                       | .912                        | 3.79                                          | 5.12                              | .346                             | 2.72                                         | 5.09                             |
| 70.0*                           | 0.515 | .308                       | 1.013                       | 3.35                                          | 5.00                              | .517                             | 3.29                                         | 5.00                             |
| 80.0*                           | 0.497 | .231                       | 1.089                       | 2.94                                          | 4.90                              | .673                             | 3.91                                         | 4.92                             |
| 90.0*                           | 0.481 | .163                       | 1.157                       | 2.93                                          | 4.81                              | .852                             | 4.99                                         | 4.85                             |
| 100                             | 0.467 | .103                       | 1.215                       | 3.39                                          | 4.73                              | +1.07                            | 7.17                                         | 4.78                             |
| 110                             | 0.462 | .0814                      | 1.235                       | 2.99                                          | 4.65                              | +1.18                            | 7.94                                         | 4.72                             |
| 160                             | 0.456 | .0555                      | 1.255                       | 1.21                                          | 4.36                              | +1.35                            | 6.84                                         | 4.48                             |
| 210                             | 0.453 | .0426                      | 1.261                       | 0.722                                         | 4.19                              | +1.47                            | 6.31                                         | 4.33                             |



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<sup>a</sup>Refer to Table 7 for reaction conditions.

Table 20. Calculated values of [BH], [BHP],  $K_s$ , " $K_I$ ", and related data for the reaction between 3-aminoacridinium cation and DNA in 0.010N  $Mg(O_2CCH_3)_2$

| Vol<br>DNA<br>( $\mu$ l) | Abs   | [BH]<br>N<br>$\times 10^5$ | [BHP]<br>N<br>$\times 10^5$ | $K_I^b$<br>N <sup>-2</sup><br>$\times 10^{-7}$ | $-\log([P_t]/_2^{-2}[BHP])$ | $\log([BHP]/[BH])$ | $K_s$<br>N <sup>-1</sup><br>$\times 10^{-4}$ | $-\log([P_t]/_3^{-1}[BHP])$ |
|--------------------------|-------|----------------------------|-----------------------------|------------------------------------------------|-----------------------------|--------------------|----------------------------------------------|-----------------------------|
| 0                        | 0.742 | 1.313                      | 0                           | 111                                            | 5.08                        | -1.11              | 1.32                                         | 5.23                        |
| 25.0                     | 0.716 | 1.215                      | .095                        | 55.0                                           | 4.77                        | -.801              | 1.33                                         | 4.92                        |
| 50.0                     | 0.693 | 1.128                      | .178                        | 36.0                                           | 4.59                        | -.624              | 1.32                                         | 4.75                        |
| 75.0**                   | 0.673 | 1.053                      | .251                        | 25.8                                           | 4.46                        | -.508              | 1.28                                         | 4.62                        |
| 100**                    | 0.657 | .992                       | .308                        | 17.5                                           | 4.28                        | -.316              | 1.33                                         | 4.44                        |
| 150**                    | 0.625 | .872                       | .422                        | 13.1                                           | 4.15                        | -.184              | 1.34                                         | 4.31                        |
| 200**                    | 0.600 | .778                       | .509                        | 10.2                                           | 4.05                        | -.090              | 1.33                                         | 4.21                        |
| 250                      | 0.581 | .707                       | .575                        | 8.40                                           | 3.97                        | -.012              | 1.32                                         | 4.13                        |
| 300                      | 0.565 | .646                       | .629                        | 7.16                                           | 3.90                        | +.056              | 1.32                                         | 4.06                        |
| 350                      | 0.551 | .594                       | .675                        | 6.09                                           | 3.84                        | +.105              | 1.29                                         | 4.01                        |
| 400                      | 0.541 | .556                       | .707                        | 4.95                                           | 3.74                        | +.210              | 1.32                                         | 3.91                        |
| 500                      | 0.520 | .477                       | .773                        | 4.07                                           | 3.66                        | +.283              | 1.30                                         | 3.83                        |
| 600*                     | 0.506 | .424                       | .814                        | 3.45                                           | 3.60                        | +.343              | 1.29                                         | 3.77                        |
| 700*                     | 0.495 | .383                       | .844                        | 3.03                                           | 3.54                        | +.401              | 1.29                                         | 3.71                        |
| 800*                     | 0.485 | .346                       | .871                        | 2.67                                           | 3.49                        | +.444              | 1.27                                         | 3.66                        |
| 900*                     | 0.478 | .319                       | .886                        |                                                |                             |                    |                                              |                             |

| Vol<br>DNA<br>( $\mu$ l) | Abs   | [BH]<br>N<br>$\times 10^5$ | [BHP]<br>N<br>$\times 10^5$ | $K_I^b$<br>N <sup>-2</sup><br>$\times 10^{-7}$ | $-\log([P_t]/^2-2[BHP])$ | $\log([BHP]/[BH])$ | $K_S$<br>N <sup>-1</sup><br>$\times 10^{-4}$ | $-\log([P_t]/_3-[BHP])$ |
|--------------------------|-------|----------------------------|-----------------------------|------------------------------------------------|--------------------------|--------------------|----------------------------------------------|-------------------------|
| 1100*                    | 0.467 | .277                       | .906                        | 2.15                                           | 3.41                     | + .515             | 1.24                                         | 3.58                    |
| 1300*                    | 0.459 | .246                       | .916                        | 1.79                                           | 3.34                     | + .571             | 1.21                                         | 3.51                    |
| 1650                     | 0.449 | .207                       | .920                        | 1.39                                           | 3.25                     | + .647             | 1.17                                         | 3.42                    |

<sup>a</sup>Refer to Table 9 for reaction conditions.

<sup>b</sup>Not a valid association constant as the intercalative process was not seen under these conditions. Included for illustrative purposes only. See text.

Table 21. Calculated values of [BH], [BHP],  $K_s$ , " $K_I$ ", and related data for the reaction between 3-aminoacridinium cation and DNA in  $6.3 \times 10^{-4}$  N  $Mg(O_2CCH_3)_2$

| Vol<br>DNA<br>( $\mu$ l) | Abs   | [BH]<br>N<br>$\times 10^5$ | [BHP]<br>N<br>$\times 10^5$ | $K_I^b$<br>N <sup>-2</sup><br>$\times 10^{-8}$ | $-\log([P_t]/_2 - 2[BHP])$ | $\log([BHP]/[BH])$ | $K_s$<br>N <sup>-1</sup><br>$\times 10^{-4}$ | $-\log([P_t]/_3 - [BHP])$ |
|--------------------------|-------|----------------------------|-----------------------------|------------------------------------------------|----------------------------|--------------------|----------------------------------------------|---------------------------|
| 0                        | 0.718 | 1.271                      | 0                           | 198                                            | 5.68                       | -1.06              | 5.01                                         | 5.76                      |
| 10.0                     | 0.692 | 1.168                      | .101                        | 65.1                                           | 5.24                       | - .664             | 4.71                                         | 5.34                      |
| 25.0                     | 0.660 | 1.042                      | .226                        | 37.8                                           | 5.01                       | - .449             | 4.72                                         | 5.12                      |
| 40.0                     | 0.632 | .931                       | .334                        | 26.0                                           | 4.85                       | - .292             | 4.74                                         | 4.97                      |
| 55.0                     | 0.608 | .837                       | .427                        | 19.6                                           | 4.73                       | - .172             | 4.80                                         | 4.85                      |
| 70.0                     | 0.587 | .750                       | .508                        | 15.6                                           | 4.63                       | - .074             | 4.85                                         | 4.76                      |
| 85.0                     | 0.569 | .683                       | .577                        | 12.8                                           | 4.55                       | + .007             | 4.86                                         | 4.68                      |
| 100                      | 0.554 | .624                       | .634                        | 9.80                                           | 4.44                       | + .119             | 4.91                                         | 4.57                      |
| 125                      | 0.533 | .542                       | .713                        | 7.80                                           | 4.34                       | + .208             | 4.91                                         | 4.48                      |
| 150                      | 0.517 | .479                       | .773                        | 6.56                                           | 4.26                       | + .289             | 4.98                                         | 4.41                      |
| 175                      | 0.503 | .424                       | .825                        | 5.62                                           | 4.20                       | + .356             | 5.02                                         | 4.34                      |
| 200                      | 0.492 | .381                       | .865                        | 4.37                                           | 4.09                       | + .469             | 5.09                                         | 4.24                      |
| 250                      | 0.475 | .314                       | .926                        | 3.49                                           | 4.00                       | + .551             | 5.04                                         | 4.15                      |
| 300                      | 0.464 | .271                       | .963                        | 2.94                                           | 3.92                       | + .624             | 5.07                                         | 4.08                      |
| 350                      | 0.455 | .236                       | .992                        | 2.54                                           | 3.86                       | + .687             | 5.09                                         | 4.02                      |
| 400                      | 0.448 |                            |                             |                                                |                            |                    |                                              |                           |

| Vol<br>DNA<br>( $\mu$ l) | Abs   | [BH]<br>N<br>$\times 10^5$ | [BHP]<br>N<br>$\times 10^5$ | $K_I^b$<br>N <sup>-2</sup><br>$\times 10^{-8}$ | $-\log([P_t]/_2 - 2[BHP])$ | $\log([BHP]/[BH])$ | $K_S$<br>N <sup>-1</sup><br>$\times 10^{-4}$ | $-\log([P_t]/_3 - [BHP])$ |
|--------------------------|-------|----------------------------|-----------------------------|------------------------------------------------|----------------------------|--------------------|----------------------------------------------|---------------------------|
| 500                      | 0.439 | .208                       | 1.014                       | 1.94                                           | 3.76                       | + .778             | 4.98                                         | 3.92                      |
| 600                      | 0.434 | .173                       | 1.037                       | 1.51                                           | 3.67                       | + .835             | 4.71                                         | 3.84                      |
| 800                      | 0.427 | .153                       | 1.046                       | 1.04                                           | 3.55                       | + .926             | 4.37                                         | 3.71                      |
| 1000                     | 0.426 | .125                       | 1.052                       | 0.69                                           | 3.45                       | + .940             | 3.64                                         | 3.62                      |

<sup>a</sup>Refer to Table 9 for reaction conditions.

<sup>b</sup>Not a valid association constant as the intercalative process was not seen under these conditions. Included for illustrative purposes only. See text.

Table 22. Percent total 3-aminoacridinium bound to DNA in the presence of various electrolytes after addition of excess DNA<sup>a</sup>

| Electrolyte <sup>b</sup>                | Ionic strength (I) <sup>c</sup> | P/D <sup>d</sup> | % of total drug bound |
|-----------------------------------------|---------------------------------|------------------|-----------------------|
| 0.15M $C_sH_2PO_4$                      | 0.14                            | 65               | 90                    |
| 0.10M $C_sH_2PO_4$                      | 0.094                           | 55               | 94                    |
| 0.010M $C_sH_2PO_4$                     | 0.010                           | 17               | 97                    |
| 0.0025M $C_sH_2PO_4$                    | 0.0028                          | 14               | 97                    |
| 0.10M $LiH_2PO_4$                       | 0.094                           | 57               | 89                    |
| 0.010M $LiH_2PO_4$                      | 0.010                           | 15               | 99                    |
| $6.3 \times 10^{-4}$ M $LiH_2PO_4$      | 0.0012                          | 11               | 98                    |
| 0.010N $Ba(O_2CCH_3)_2$                 | 0.017                           | 103              | 77                    |
| $6.3 \times 10^{-4}$ N $Ba(O_2CCH_3)_2$ | 0.0027                          | 47               | 90                    |
| 0.010N $Mg(O_2CCH_3)_2$                 | 0.017                           | 104              | 70                    |
| $6.3 \times 10^{-4}$ N $Mg(O_2CCH_3)_2$ | 0.0034                          | 65               | 90                    |

<sup>a</sup>pH 5.90, T = 25.0°C.

<sup>b</sup>Concentrations before any DNA added.

<sup>c</sup>In equivalents per liter for the solution containing the volume of DNA necessary for the P/D ratio listed.

<sup>d</sup>Total moles of DNA phosphate per total moles of 3-AA.

Table 23. Initial and final slopes of plots of  $\log([BHP]/[BH])$  vs.  $\log([P_t/m] - q[BHP])$  in various concentrations of  $C_5H_2PO_4$  and  $Mg(O_2CCH_3)_2$

| Electrolyte                             | Initial slope        |                       | Final slope          |                       |
|-----------------------------------------|----------------------|-----------------------|----------------------|-----------------------|
|                                         | <u>I<sup>b</sup></u> | <u>II<sup>c</sup></u> | <u>I<sup>b</sup></u> | <u>II<sup>c</sup></u> |
| 0.15M $C_5H_2PO_4$                      | 1.14                 | 1.16                  | 1.14                 | 1.16                  |
| 0.010M $C_5H_2PO_4$                     | 1.07                 | 1.23                  | 1.68                 | 1.88                  |
| 0.0025M $C_5H_2PO_4$                    | 1.10                 | 1.14                  | 1.71                 | 2.16                  |
| 0.010N $Mg(O_2CCH_3)_2$                 | 0.96                 | 0.97                  | 0.96                 | 0.97                  |
| $6.3 \times 10^{-4}$ N $Mg(O_2CCH_3)_2$ | 0.98                 | 1.0                   | 0.98                 | 1.0                   |

<sup>a</sup>Titration done at 25.0°C, pH 5.90.

<sup>b</sup> $\log([BHP]/[BH])$  vs.  $\log([P_t/2] - 2[BHP])$ .

<sup>c</sup> $\log([BHP]/[BH])$  vs.  $\log([P_t/3] - [BHP])$ .

Table 24. Apparent equilibrium association constants for surface,  $K_s$ , and intercalative,  $K_I$ , binding of 3-aminoacridinium to DNA,  $\text{LiH}_2\text{PO}_4$  as supporting electrolyte.<sup>a</sup>

| I<br>(ave) | $K_s \text{ M}^{-1}$<br>$\times 10^{-4}$ | $\sigma(n)^b$<br>$\times 10^{-4}$ | I<br>(ave) | $K_I \text{ M}^{-2}$<br>$\times 10^{-9}$ | $\sigma(n)^b$<br>$\times 10^{-9}$ |
|------------|------------------------------------------|-----------------------------------|------------|------------------------------------------|-----------------------------------|
| .099       | 2.66                                     | 0.112(4)                          | .097       | 0.233                                    | 0.044(4)                          |
| .10        | 3.03                                     | 0.181(4)                          | .098       | 0.299                                    | 0.066(4)                          |
|            | <u>2.85</u>                              |                                   |            | <u>0.266</u>                             |                                   |
| .050       | 4.28                                     | 0.333(4)                          | .049       | 0.989                                    | 0.118(4)                          |
| .050       | 3.54                                     | 0.325(3)                          | .049       | 0.565                                    | 0.116(4)                          |
|            | <u>3.91</u>                              |                                   |            | <u>0.777</u>                             |                                   |
| .025       | 5.40                                     | 0.309(3)                          | .025       | 2.41                                     | 0.366(4)                          |
| .010       | 9.01                                     | 0.888(3)                          | .010       | 8.35                                     | .661(3)                           |
| .010       | 11.0                                     | 0.938(3)                          | .010       | 8.89                                     | .974(5)                           |
|            | <u>10.0</u>                              |                                   |            | <u>8.62</u>                              |                                   |
| .0050      | 13.5                                     | 0.404(3)                          | .0051      | 15.8                                     | 3.73(4)                           |
| .0050      | 21.7                                     | 0.071(2)                          | .0051      | 34.6                                     | 10.8(3)                           |
| .0050      | 12.4                                     | 0.416(3)                          | .0051      | 13.8                                     | 1.06(4)                           |
|            | <u>15.9</u>                              |                                   |            | <u>14.8</u>                              |                                   |
| .0025      | 27.1                                     | 0.990(2)                          | .0026      | 78.2                                     | 29.3(2)                           |
| .0025      | 16.0                                     | 1.56(4)                           | .0026      | 22.8                                     | 2.81(4)                           |
|            | <u>21.6</u>                              |                                   |            | <u>50.5</u>                              |                                   |



| I<br>(ave) | $K_S M^{-1}$<br>$\times 10^{-4}$ | $\sigma(n)^b$<br>$\times 10^{-4}$ | I<br>(ave) | $K_I M^{-2}$<br>$\times 10^{-9}$ | $\sigma(n)^b$<br>$\times 10^{-9}$ |
|------------|----------------------------------|-----------------------------------|------------|----------------------------------|-----------------------------------|
| .00066     | 16.4                             | 1.53(3)                           | .0074      | 53.7                             | 4.18(4)                           |
| .00066     | 22.7                             | 1.50(3)                           | .0073      | 80.5                             | 7.00(3)                           |
|            | 19.6                             |                                   |            | 67.1                             |                                   |

<sup>a</sup> Constants based on molar concentrations of species,  $T = 25.0^\circ\text{C}$ , pH 5.90.

<sup>b</sup>  $\sigma$  is the standard deviation from the mean for the population, n.

Table 25. Apparent equilibrium association constants for surface,  $K_s$ , and intercalative,  $K_I$ , binding of 3-aminoacridinium to DNA,  $\text{NaH}_2\text{PO}_4$  as supporting electrolyte<sup>a</sup>

| I<br>(ave) | $K_s \text{ M}^{-1}$<br>$\times 10^{-4}$ | $\sigma(n)$<br>$\times 10^{-4}$ | I<br>(ave) | $K_I \text{ M}^{-2}$<br>$\times 10^{-9}$ | $\sigma(n)$<br>$\times 10^{-9}$ |
|------------|------------------------------------------|---------------------------------|------------|------------------------------------------|---------------------------------|
| .100       | 3.49                                     | 0.221(4)                        | .098       | 0.518                                    | 0.0825(3)                       |
| .099       | 3.31                                     | 0.155(4)                        | .098       | 0.363                                    | 0.0817(5)                       |
|            | 3.40                                     |                                 |            | 0.441                                    |                                 |
| .050       | 5.23                                     | 0.268(3)                        | .050       | 1.67                                     | 0.234(4)                        |
| .050       | 5.42                                     | 0.258(3)                        | .050       | 1.56                                     | 0.223(3)                        |
|            | 5.33                                     |                                 |            | 1.62                                     |                                 |
| .025       | 7.51                                     | 0.312(3)                        | .025       | 4.59                                     | 0.797(4)                        |
| .025       | 7.96                                     | 0.284(3)                        | .025       | 5.55                                     | 0.875(4)                        |
|            | 7.74                                     |                                 |            | 5.07                                     |                                 |
| .010       | 12.7                                     | 0.265(3)                        | .010       | 16.4                                     | 1.20(2)                         |
| .010       | 12.8                                     | 0.071(2)                        | .010       | 17.0                                     | 1.27(2)                         |
|            | 12.8                                     |                                 |            | 16.7                                     |                                 |
| .0050      | 27.8                                     | 2.59(3)                         | .0051      | 59.8                                     | 10.3(2)                         |
| .0025      | 31.2                                     | 7.61(3)                         | .0026      | 75.0                                     | 22.7(2)                         |

<sup>a</sup>Constants based on molar concentrations of species.  $T = 25.0^\circ\text{C}$ , pH 5.90.

<sup>b</sup> $\sigma$  is the standard deviation from the mean for the population, n.

Table 20. Apparent equilibrium association constants for suriace,  $K_s$ , and intercalative,  $K_i$ , binding of 3-aminoacridinium to DNA,  $KH_2PO_4$  as supporting electrolyte<sup>a</sup>

| I<br>(ave) | $K_s \text{ M}^{-1}$<br>$\times 10^{-4}$ | $\sigma(n)^b$<br>$\times 10^{-4}$ | I<br>(ave) | $K_i \text{ M}^{-2}$<br>$\times 10^{-9}$ | $\sigma(n)^b$<br>$\times 10^{-9}$ |
|------------|------------------------------------------|-----------------------------------|------------|------------------------------------------|-----------------------------------|
| .10        | 3.48                                     | 0.252(4)                          | .098       | .478                                     | 0.122(6)                          |
| .10        | 4.33                                     | 0.147(4)                          | .098       | .644                                     | 0.135(6)                          |
|            | <u>3.91</u>                              |                                   |            | <u>.561</u>                              |                                   |
| .050       | 6.12                                     | 0.266(5)                          | .050       | 1.84                                     | 0.285(5)                          |
| .050       | 5.97                                     | 0.552(5)                          | .050       | 2.07                                     | 0.306(5)                          |
|            | <u>6.05</u>                              |                                   |            | <u>1.96</u>                              |                                   |
| .025       | 9.48                                     | 0.348(3)                          | .025       | 5.44                                     | 0.502(5)                          |
| .025       | 8.85                                     | 0.350(3)                          | .025       | 5.25                                     | 0.389(5)                          |
|            | <u>9.17</u>                              |                                   |            | <u>5.35</u>                              |                                   |
| .010       | 13.6                                     | 0.594(4)                          | .010       | 14.7                                     | 1.14(6)                           |
| .010       | 13.6                                     | 0.793(3)                          | .010       | 15.0                                     | 0.997(4)                          |
|            | <u>13.6</u>                              |                                   |            | <u>14.9</u>                              |                                   |
| .0050      | 11.0                                     | 0.583(4)                          | .0051      | 11.8                                     | 0.707(4)                          |
| .0050      | 23.0                                     | 3.75(3)                           | .0051      | 26.4                                     | 3.06(4)                           |
|            | <u>17.0</u>                              |                                   |            | <u>19.1</u>                              |                                   |
| .00066     | 43.1                                     | 8.54(3)                           | .00072     | 176                                      | 29.1(3)                           |
| .00066     | 30.0                                     | 4.43(4)                           | .00073     | 112                                      | 21.0(5)                           |
|            | <u>36.6</u>                              |                                   |            | <u>144</u>                               |                                   |

<sup>a</sup> Constants based on molar concentrations of species.  $T = 25.0^\circ\text{C}$ , pH 5.90.

$\sigma$  is the standard deviation from the mean for the population,  $n$ .

Table 27. Apparent equilibrium association constants for surface,  $K_s$ , and intercalative,  $K_I$ , binding of 3-aminoacridinium to DNA,  $\text{KO}_2\text{CCH}_3$  as supporting electrolyte<sup>a</sup>

| I<br>(ave) | $K_s \text{ M}^{-1}$<br>$\times 10^{-4}$ | $\sigma(n)^b$<br>$\times 10^{-4}$ | I<br>(ave) | $K_I \text{ M}^{-2}$<br>$\times 10^{-9}$ | $\sigma(n)^b$<br>$\times 10^{-9}$ |
|------------|------------------------------------------|-----------------------------------|------------|------------------------------------------|-----------------------------------|
| .15        | 2.64                                     | 0.249(4)                          | .14        | .204                                     | 0.047(6)                          |
| .15        | 2.76                                     | 0.080(4)                          | .15        | .247                                     | 0.044(4)                          |
|            | <u>2.70</u>                              |                                   |            | <u>.226</u>                              |                                   |
| .050       | 5.70                                     | 0.547(4)                          | .050       | 1.96                                     | 0.201(5)                          |
| .050       | 5.37                                     | 0.269(4)                          | .050       | 1.86                                     | 0.217(5)                          |
|            | <u>5.54</u>                              |                                   |            | <u>1.91</u>                              |                                   |
| .025       | 8.92                                     | 1.12(3)                           | .025       | 5.75                                     | 0.589(5)                          |
| .025       | 7.40                                     | 0.379(3)                          | .025       | 3.64                                     | 0.481(5)                          |
|            | <u>8.16</u>                              |                                   |            | <u>4.70</u>                              |                                   |
| .0050      | 26.4                                     | 3.33(4)                           | .0051      | 39.8                                     | 7.39(4)                           |
| .0050      | 25.5                                     | 7.14(2)                           | .0051      | 29.6                                     | 6.20(3)                           |
| .0050      | 15.8                                     | 1.13(3)                           | .0051      | 27.2                                     | 2.59(4)                           |
|            | <u>22.6</u>                              |                                   |            | <u>32.2</u>                              |                                   |
| .0025      | 19.8                                     | 1.58(4)                           | .0026      | 51.8                                     | 3.69(4)                           |
| .0025      | 19.2                                     | 0.494(2)                          | .0026      | 49.6                                     | 6.21(4)                           |
|            | <u>19.5</u>                              |                                   |            | <u>50.7</u>                              |                                   |
| .00066     | 17.0                                     | 1.51(5)                           | .00075     | 55.7                                     | 5.74(4)                           |
| .00066     | 11.2                                     | 1.25(3)                           | .00075     | 23.0                                     | 1.36(3)                           |
|            | <u>14.1</u>                              |                                   |            | <u>39.4</u>                              |                                   |

<sup>a</sup>Constants based on molar concentrations of species.  $T = 25.0^\circ\text{C}$ , pH 5.90.

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$b_{\sigma}$  is the standard deviation from the mean for the population,  $n$ .

Table 28. Apparent equilibrium association constants for surface,  $K_s$ , and intercalative,  $K_I$ , binding of 3-aminoacridinium to DNA,  $RbH_2PO_4$  as supporting electrolyte<sup>a</sup>

| I<br>(ave) | $K_s \text{ M}^{-1}$<br>$\times 10^{-4}$ | $\sigma(n)^b$<br>$\times 10^{-4}$ | I<br>(ave) | $K_I \text{ M}^{-2}$<br>$\times 10^{-9}$ | $\sigma(n)^b$<br>$\times 10^{-9}$ |
|------------|------------------------------------------|-----------------------------------|------------|------------------------------------------|-----------------------------------|
| .10        | 3.95                                     | 0.162(4)                          | .098       | 0.566                                    | 0.118(6)                          |
| .10        | 3.73                                     | 0.397(4)                          | .098       | 0.629                                    | 0.134(8)                          |
|            | <u>3.84</u>                              |                                   |            | <u>0.598</u>                             |                                   |
| .050       | 5.66                                     | 0.619(4)                          | .049       | 1.98                                     | 0.167(4)                          |
| .050       | 6.45                                     | 0.205(2)                          | .049       | 2.11                                     | 0.317(6)                          |
|            | <u>6.06</u>                              |                                   |            | <u>2.05</u>                              |                                   |
| .025       | 9.17                                     | 0.197(3)                          | .025       | 5.28                                     | 0.606(5)                          |
| .025       | 10.9                                     | 0.283(2)                          | .025       | 6.78                                     | 0.445(5)                          |
| .025       | 10.2                                     | 0.601(2)                          | .025       | 6.70                                     | 0.605(4)                          |
|            | <u>10.1</u>                              |                                   |            | <u>6.25</u>                              |                                   |
| .010       | 11.5                                     | 1.70(2)                           | .010       | 17.8                                     | 1.31(3)                           |
| .010       | 17.5                                     | 2.83(2)                           | .010       | 26.4                                     | 2.02(5)                           |
|            | <u>14.5</u>                              |                                   |            | <u>22.1</u>                              |                                   |
| .0050      | 13.8                                     | 1.67(4)                           | .0051      | 18.5                                     | 1.65(5)                           |
| .0050      | 6.74 <sup>c</sup>                        | 2.23(3)                           | .0051      | 67.4*                                    | 22.3(3)                           |
| .0050      | 15.7                                     | 0.902(4)                          | .0051      | 27.3                                     | 2.94(5)                           |
|            | <u>14.8</u>                              |                                   |            | <u>22.9</u>                              |                                   |

Table 28 (Continued)

| I<br>(ave) | $K_s \text{ M}^{-1}$<br>$\times 10^{-4}$ | $\sigma(n)^b$<br>$\times 10^{-4}$ | I<br>(ave) | $K_I \text{ M}^{-2}$<br>$\times 10^{-9}$ | $\sigma(n)^n$<br>$\times 10^{-9}$ |
|------------|------------------------------------------|-----------------------------------|------------|------------------------------------------|-----------------------------------|
| .0025      | 16.2                                     | 0.850(3)                          | .00026     | 28.2                                     | 2.08(4)                           |
| .0025      | 16.6                                     | 0.379(3)                          | .00026     | 24.8                                     | 2.54(5)                           |
|            | <u>16.4</u>                              |                                   |            | <u>26.5</u>                              |                                   |

<sup>a</sup>Constants based on molar concentrations of species. T = 25.0°C, pH 5.90.

<sup>b</sup> $\sigma$  is the standard deviation from the mean for the population, n.

<sup>c</sup>Value not included in average.

Table 29. Apparent equilibrium association constants for surface,  $K_s$ , and intercalative,  $K_I$ , binding of 3-aminoacridinium to DNA,  $\text{C}_5\text{H}_2\text{PO}_4$  as supporting electrolyte<sup>a</sup>

| I<br>(ave) | $K_s \text{ M}^{-1}$<br>$\times 10^{-4}$ | $\sigma(n)^b$<br>$\times 10^{-4}$ | I<br>(ave) | $K_I \text{ M}^{-2}$<br>$\times 10^{-9}$ | $\sigma(n)^b$<br>$\times 10^{-9}$ |
|------------|------------------------------------------|-----------------------------------|------------|------------------------------------------|-----------------------------------|
| .15        | 2.30                                     | 0.154(6)                          |            |                                          |                                   |
| .15        | 2.59                                     | 0.275(6)                          |            |                                          |                                   |
|            | <u>2.45</u>                              |                                   |            |                                          |                                   |
| .10        | 3.49                                     | 0.212(6)                          | .098       | 0.414                                    | 0.097(6)                          |
| .10        | 4.99                                     | 0.610(5)                          | .098       | 0.627                                    | 0.115(4)                          |
|            | <u>4.24</u>                              |                                   |            | <u>0.521</u>                             |                                   |
| .050       | 4.21                                     | 0.534(5)                          | .049       | 0.965                                    | 0.022(5)                          |
| .050       | 5.99                                     | 0.320(6)                          | .050       | 1.71                                     | 0.176(5)                          |
|            | <u>5.10</u>                              |                                   |            | <u>1.34</u>                              |                                   |
| .025       | 8.88                                     | 0.384(5)                          | .025       | 4.13                                     | 0.429(5)                          |
| .025       | 5.40                                     | 0.384(5)                          | .025       | 1.34                                     | 0.172(5)                          |
|            | <u>7.14</u>                              |                                   |            | <u>2.74</u>                              |                                   |
| .010       | 11.7                                     | 0.390(5)                          | .010       | 9.97                                     | 1.21(5)                           |
| .010       | 12.3                                     | 1.37(4)                           | .010       | 7.55                                     | 0.588(4)                          |
|            | <u>12.0</u>                              |                                   |            | <u>8.76</u>                              |                                   |
| .0025      | 19.6                                     | 2.50(4)                           | .0026      | 34.2                                     | 5.09(5)                           |
| .0025      | 18.5                                     | 3.44(4)                           | .0026      | 15.9                                     | 2.91(5)                           |
|            | <u>19.1</u>                              |                                   |            | <u>25.1</u>                              |                                   |

<sup>c</sup>



Table 29 (Continued)

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<sup>a</sup>Constants based on molar concentrations of species.  $T = 25.0^{\circ}\text{C}$ , pH 5.90.

<sup>b</sup> $\sigma$  is the standard deviation from the mean for the population, n.

<sup>c</sup>Intercalative binding not seen due to high  $\text{Cs}^+$  concentration.

Table 30. Apparent equilibrium association constants for the surface,  $K_s$ , and intercalative,  $K_I$ , binding of 3-aminoacridinium to DNA,  $(CH_3)_4NH_2PO_4$  as supporting electrolyte<sup>a</sup>

| I<br>(ave) | $K_s \text{ M}^{-1}$<br>$\times 10^{-4}$ | $\sigma(n)^b$<br>$\times 10^{-4}$ | I<br>(ave) | $K_I \text{ M}^{-2}$<br>$\times 10^{-9}$ | $\sigma(n)^b$<br>$\times 10^{-9}$ |
|------------|------------------------------------------|-----------------------------------|------------|------------------------------------------|-----------------------------------|
| .10        | 6.75                                     | 0.317(3)                          | .099       | 2.39                                     | 0.235(5)                          |
| .10        | 5.35                                     | 0.296(4)                          | .099       | 1.75                                     | 0.226(4)                          |
|            | <u>6.05</u>                              |                                   |            | <u>2.07</u>                              |                                   |
| .050       | 6.92                                     | 0.578(2)                          | .050       | 3.86                                     | 0.059(4)                          |
| .050       | 7.58                                     | 0.288(4)                          | .050       | 4.06                                     | 0.169(3)                          |
|            | <u>7.25</u>                              |                                   |            | <u>3.96</u>                              |                                   |
| .025       | 10.5                                     | 0.495(2)                          | .025       | 4.19                                     | 0.577(4)                          |
| .025       | 8.80                                     | 4.65(4)                           | .025       | 8.33                                     | 0.417(3)                          |
| .025       | 13.3                                     | 0.0(2)                            | .025       | 12.0                                     | 1.07(4)                           |
| .025       | 10.1                                     | 0.630(3)                          | .025       | 8.78                                     | 0.849(3)                          |
|            | <u>10.7</u>                              |                                   |            | <u>8.33</u>                              |                                   |
| .010       | 13.1                                     | 0.785(3)                          | .010       | 21.7                                     | 3.01(3)                           |
| .010       | 17.6                                     | 2.50(3)                           | .010       | 36.3                                     | 0.905(2)                          |
| .010       | 19.0                                     | 2.34(2)                           | .010       | 30.9                                     | 4.06(4)                           |
|            | <u>16.6</u>                              |                                   |            | <u>29.6</u>                              |                                   |
| .0050      | 27.0                                     | 0.712(3)                          | .0051      | 51.7                                     | 6.59(2)                           |
| .0050      | 25.3                                     | 0.488(2)                          | .0051      | 74.4                                     | 14.0(4)                           |
|            | <u>26.2</u>                              |                                   | .0051      | 53.3                                     | 10.8(4)                           |
|            |                                          |                                   |            | <u>59.8</u>                              |                                   |

Table 30 (Continued)

| I<br>(ave) | $K_S \text{ M}^{-1}$<br>$\times 10^{-4}$ | $\sigma(n)^b$<br>$\times 10^{-4}$ | I<br>(ave) | $K_I \text{ M}^{-2}$<br>$\times 10^{-9}$ | $\sigma(n)^b$<br>$\times 10^{-9}$ |
|------------|------------------------------------------|-----------------------------------|------------|------------------------------------------|-----------------------------------|
| .0025      | 21.3                                     | 1.18(3)                           |            |                                          |                                   |
| .0025      | 100                                      | 9.00(2)                           | .0026      | 63.9                                     | 7.77(4)                           |
| .0025      | 50.4                                     | 32.0(2)                           | .0026      | 109                                      | 4.48(4)                           |
|            | <u>57.2</u>                              |                                   | .0026      | <u>55.1</u>                              | <u>11.5(4)</u>                    |
|            |                                          |                                   |            | 76.0                                     |                                   |

<sup>a</sup>Constants based on molar concentrations of species.  $T = 25.0^\circ\text{C}$ , pH 5.90.

<sup>b</sup> $\sigma$  is the standard deviation from the mean for the population, n.

Table 31. Apparent equilibrium association constants,  $K_s$ , for surface binding of 3-aminoacridinium to DNA,  $Mg(O_2CCH_3)_2$  and  $Ca(O_2CCH_3)_2$  as supporting electrolytes<sup>a</sup>

| $Mg(O_2CCH_3)_2$ |                                       |                                 |            | $Ca(O_2CCH_3)_2$                      |                                 |            |                                       |
|------------------|---------------------------------------|---------------------------------|------------|---------------------------------------|---------------------------------|------------|---------------------------------------|
| I<br>(ave)       | $K_s$<br>$N^{-1}$<br>$\times 10^{-4}$ | $\sigma(n)$<br>$\times 10^{-4}$ | I<br>(ave) | $K_s$<br>$N^{-1}$<br>$\times 10^{-4}$ | $\sigma(n)$<br>$\times 10^{-4}$ | I<br>(ave) | $K_s$<br>$N^{-1}$<br>$\times 10^{-4}$ |
| .015             | 1.32                                  | 0.031(5)                        | .037       | .968                                  | 0.035(5)                        |            |                                       |
| .015             | 1.32                                  | 0.026(4)                        | .015       | 1.43                                  | 0.032(4)                        |            |                                       |
|                  | <u>1.32</u>                           |                                 |            |                                       |                                 |            |                                       |
| .0077            | 2.08                                  | 0.054(4)                        | .0077      | 1.68                                  | 0.042(5)                        |            |                                       |
| .0077            | 2.04                                  | 0.073(4)                        | .0076      | 2.15                                  | 0.107(4)                        |            |                                       |
|                  | <u>2.06</u>                           |                                 |            | <u>1.92</u>                           |                                 |            |                                       |
| .0039            | 2.76                                  | 0.026(4)                        | .0040      | 2.41                                  | 0.034(5)                        |            |                                       |
| .0039            | 2.76                                  | 0.079(4)                        | .0039      | 2.34                                  | 0.024(5)                        |            |                                       |
|                  | <u>2.76</u>                           |                                 |            | <u>2.38</u>                           |                                 |            |                                       |
| .0021            | 4.27                                  | 0.025(4)                        | .0021      | 3.48                                  | 0.520(4)                        |            |                                       |
| .0021            | 3.50                                  | 0.082(4)                        | .0022      | 2.45                                  | 0.036(5)                        |            |                                       |
|                  | <u>3.89</u>                           |                                 |            | <u>2.97</u>                           |                                 |            |                                       |
| .0011            | 4.74                                  | 0.040(4)                        | .0011      | 2.98                                  | 0.078(5)                        |            |                                       |
| .0011            | 4.90                                  | 0.170(2)                        | .0011      | 3.54                                  | 0.201(5)                        |            |                                       |
|                  | <u>4.82</u>                           |                                 |            | <u>3.26</u>                           |                                 |            |                                       |
|                  |                                       |                                 | .00012     | 8.42                                  | 0.153(4)                        |            |                                       |

Table 31 (Continued)

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<sup>a</sup>Constants based on molar concentrations of species.  $T = 25.0^{\circ}\text{C}$ , pH 5.90.

<sup>b</sup> $\sigma$  is the standard deviation from the mean for the population, n.

Table 32. Apparent equilibrium association constants,  $K_S$ , for the surface binding of 3-aminoacridinium to DNA,  $Sr(O_2CCH_3)_2$  and  $Ba(O_2CCH_3)_2$  as supporting electrolytes<sup>a</sup>

| I<br>(ave) | $K_S N^{-1}$<br>$\times 10^{-4}$ | $\sigma(n)$<br>$\times 10^{-4}$ | I<br>(ave) | $K_S N^{-1}$<br>$\times 10^{-4}$ | $\sigma(n)$<br>$\times 10^{-4}$ |
|------------|----------------------------------|---------------------------------|------------|----------------------------------|---------------------------------|
| .037       | 1.03                             | 0.018(4)                        | -          | -                                | -                               |
| .037       | <u>1.03</u>                      | 0.013(4)                        |            |                                  |                                 |
|            | 1.03                             |                                 |            |                                  |                                 |
| .015       | 1.64                             | 0.044(5)                        | .015       | 1.78                             | 0.021(5)                        |
| .015       | <u>1.65</u>                      | 0.052(5)                        | .015       | <u>1.74</u>                      | 0.042(5)                        |
|            | 1.65                             |                                 |            | 1.76                             |                                 |
| .0076      | 3.64                             | 0.752(4)                        | .0077      | 2.42                             | 0.144(4)                        |
| .0077      | 2.15                             | 0.021(5)                        | .0076      | 2.60                             | 0.044(4)                        |
| .0077      | <u>2.57</u>                      | 0.048(5)                        |            | <u>2.51</u>                      |                                 |
|            | 2.36                             |                                 |            |                                  |                                 |
| .0039      | 2.90                             | 0.381(4)                        | .0039      | 3.26                             | 0.047(5)                        |
| .0039      | <u>2.94</u>                      | 0.209(4)                        | .0039      | <u>3.34</u>                      | 0.037(4)                        |
|            | 2.92                             |                                 |            | 3.30                             |                                 |
| .0020      | 4.05                             | 0.087(3)                        | .0020      | 4.99                             | 0.161(3)                        |
| .0020      | <u>4.91</u>                      | 0.142(4)                        | .0020      | <u>3.93</u>                      | 0.132(4)                        |
|            | 4.48                             |                                 |            | 4.46                             |                                 |
|            |                                  |                                 | .0010      | 6.30                             | 0.312(3)                        |
|            |                                  |                                 | .0010      | <u>5.73</u>                      | 0.091(4)                        |
|            |                                  |                                 |            | 6.02                             |                                 |

Table 32 (Continued)

| I<br>(ave) | $K_s \text{ N}^{-1}$<br>$\times 10^{-4}$ | $\sigma(n)$<br>$\times 10^{-4}$ | I<br>(ave)           | $K_s \text{ N}^{-1}$<br>$\times 10^{-4}$ | $\sigma(n)$<br>$\times 10^{-4}$ |
|------------|------------------------------------------|---------------------------------|----------------------|------------------------------------------|---------------------------------|
|            |                                          |                                 | 8.3 $\times 10^{-5}$ | 12.1                                     | 0.469(4)                        |
|            |                                          |                                 | 11 $\times 10^{-5}$  | 6.03                                     | 0.160(4)                        |
|            |                                          |                                 | 8.3 $\times 10^{-5}$ | 13.7                                     | 1.24(4)                         |
|            |                                          |                                 |                      | <u>12.9</u>                              |                                 |

<sup>a</sup>Constants based on molar concentrations of species.  $T = 25.0^\circ\text{C}$ , pH 5.90.

<sup>b</sup> $\sigma$  is the standard deviation from the mean for the population, n.

Table 33. Logarithms of the apparent surface binding association constants for the binding of 3-aminoacridinium cation to DNA, corrected for ion activities<sup>a</sup>

| Init.<br>Conc.<br>of<br>Elec-<br>trolyte<br>(M) | $\text{LiH}_2\text{PO}_4$ | $\text{NaH}_2\text{PO}_4$ | $\text{KH}_2\text{PO}_4$ | $\text{KO}_2\text{CCH}_3$ | $\text{RbH}_2\text{PO}_4$ | $\text{CsH}_2\text{PO}_4$ | $(\text{CH}_3)_4\text{NH}_2\text{PO}_4$ |
|-------------------------------------------------|---------------------------|---------------------------|--------------------------|---------------------------|---------------------------|---------------------------|-----------------------------------------|
|                                                 | $\xi_s$                   | $\log K'_s$               | $\xi_s$                  | $\log K'_s$               | $\xi_s$                   | $\log K'_s$               | $\xi_s$                                 |
| 0.15                                            | -                         | -                         | -                        | 5.31                      | 5.156                     | -                         | -                                       |
| .10                                             | 4.28                      | 5.086                     | 4.22                     | -                         | 4.20                      | 5.104                     | 4.25                                    |
| .050                                            | 3.12                      | 5.085                     | 3.09                     | 5.236                     | 3.09                      | 5.250                     | 5.410                                   |
| .025                                            | 2.51                      | 5.132                     | 2.38                     | 5.271                     | 2.38                      | 5.198                     | 5.352                                   |
| .010                                            | 1.82                      | 5.259                     | 1.82                     | 5.339                     | 1.82                      | 5.231                     | 5.407                                   |
| .0050                                           | 1.56                      | 5.392                     | 1.56                     | 5.393                     | 1.56                      | 5.338                     | 5.478                                   |
| .0025                                           | 1.38                      | 5.473                     | 1.38                     | 5.423                     | 1.38                      | 5.362                     | 5.610                                   |
| .0013                                           | 1.26                      | 5.392                     | -                        | 1.39                      | 1.38                      | 5.420                     | 5.898                                   |
| $6.3 \times 10^{-4}$                            | -                         | -                         | -                        | -                         | 1.26                      | -                         | -                                       |
|                                                 | -                         | -                         | 1.19                     | 5.224                     | 1.19                      | -                         | -                                       |



Table 33 (Continued)

| Init.<br>Conc.<br>of<br>Elec-<br>trolyte<br>(N) | Mg(O <sub>2</sub> CCH <sub>3</sub> ) <sub>2</sub> |             | Ca(O <sub>2</sub> CCH <sub>3</sub> ) <sub>2</sub> |             | Sr(O <sub>2</sub> CCH <sub>3</sub> ) <sub>2</sub> |             | Ba(O <sub>2</sub> CCH <sub>3</sub> ) <sub>2</sub> |             |
|-------------------------------------------------|---------------------------------------------------|-------------|---------------------------------------------------|-------------|---------------------------------------------------|-------------|---------------------------------------------------|-------------|
|                                                 | $\xi_s$                                           | $\log K_s'$ | $\xi_s$                                           | $\log K_s'$ | $\xi_s$                                           | $\log K_s'$ | $\xi_s$                                           | $\log K_s'$ |
| 0.025                                           | -                                                 | -           | 2.51                                              | 4.386       | 2.50                                              | 4.410       | -                                                 | -           |
| 0.010                                           | 1.94                                              | 4.409       | 1.93                                              | 4.441       | 1.93                                              | 4.502       | 1.93                                              | 4.532       |
| 0.0050                                          | 1.66                                              | 4.534       | 1.66                                              | 4.501       | 1.65                                              | 4.592       | 1.65                                              | 4.619       |
| 0.0025                                          | 1.47                                              | 4.608       | 1.46                                              | 4.542       | 1.47                                              | 4.631       | 1.47                                              | 4.685       |
| 0.0013                                          | 1.32                                              | 4.711       | 1.32                                              | 4.594       | 1.31                                              | 4.784       | 1.31                                              | 4.768       |
| 6.3 x 10 <sup>-4</sup>                          | 1.24                                              | 4.776       | 1.24                                              | 4.606       | -                                                 | -           | 1.233                                             | 4.870       |

<sup>a</sup> Ion size parameters,  $d \times 10^8$ , from reference (87) are: Li<sup>+</sup>(8); Na<sup>+</sup>(4); K<sup>+</sup>(3); Rb<sup>+</sup>, Cs<sup>+</sup>(2.5); (CH<sub>3</sub>)<sub>4</sub>N<sup>+</sup>, H<sub>2</sub>PO<sub>4</sub><sup>-</sup>(4.5); Sr<sup>++</sup>, Ba<sup>++</sup>(5); Ca<sup>++</sup>(6); and Mg<sup>++</sup>(8). Values estimated for BH, BHP, and P are 3, 4, and 4 (x 10<sup>8</sup>), respectively.

Table 34. Equilibrium association constants for the binding of 3-aminoacridinium to DNA,  $K_S''$ , and for the binding of alkali and alkaline earth metal cations to DNA,  $K_M^a$

| metal           | Alkali metals <sup>b</sup>         |                   |                  |                  |                             |
|-----------------|------------------------------------|-------------------|------------------|------------------|-----------------------------|
|                 | $\text{Na}^+$                      | $\text{K}^+$      | $\text{Rb}^+$    | $\text{Cs}^+$    | $(\text{CH}_3)_4\text{N}^+$ |
| metal conc. (M) | 0.10-<br>0.025                     | 0.050-<br>0.010   | 0.050-<br>0.010  | 0.050-<br>0.025  | 0.10-<br>0.025              |
| $\log K_S'$     | 5.158-<br>5.264                    | 5.271-<br>5.393   | 5.272-<br>5.420  | 5.198-<br>5.420  | 5.410-<br>5.407             |
| $K_M$           | 4.06                               | 8.82              | 11.3             | 14.6             | -0.09                       |
| $\log K_S''$    | 5.31                               | 5.43              | 5.47             | 5.44             | 5.41                        |
| metal           | Alkaline earth metals <sup>c</sup> |                   |                  |                  |                             |
|                 | $\text{Mg}^{++}$                   | $\text{Ca}^{++}$  | $\text{Sr}^{++}$ | $\text{Ba}^{++}$ |                             |
| metal conc. (N) | 0.010-<br>0.0025                   | 0.0050-<br>0.0013 | 0.010-<br>0.0025 | 0.010-<br>0.0025 |                             |
| $\log K_S'$     | 4.409-<br>4.608                    | 4.501-<br>4.594   | 4.410-<br>4.631  | 4.532-<br>4.685  |                             |
| $K_M$           | 96.1                               | 70.5              | 114.             | 65.5             |                             |
| $\log K_S''$    | 4.70                               | 4.63              | 4.74             | 4.75             |                             |

<sup>a</sup>Calculated using equation (3-5). All reactions at  $25.0 \pm 0.1^\circ\text{C}$ , pH 5.9. For DNA and 3-AA reactant concentrations and other experimental parameters, refer to the appropriate tables for the specific metals.

Table 34 (Continued)

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<sup>b</sup>Counteranion,  $\text{H}_2\text{PO}_4^-$

<sup>c</sup>Counteranion,  $\text{CH}_3\text{COO}^-$



Table 36. Thermodynamic parameters for the surface and intercalative binding reactions between 3-aminoacridinium and 7-aminoquinolinium cations to DNA

| cation                       | Log K<br>(Kcal)<br>298°K | $\Delta G^\circ$<br>(Kcal)<br>298°K | $\Delta H^\circ$<br>(Kcal) | $\Delta S^\circ$<br>(e.u.) |
|------------------------------|--------------------------|-------------------------------------|----------------------------|----------------------------|
| <u>surface binding</u>       |                          |                                     |                            |                            |
| 3-aminoacridinium            | 5.39                     | -7.35                               | -4.90 ± 1.3                | 8.22 ± 1.7                 |
| 7-aminoquinolinium           | 4.45                     | -6.07                               | -5.13 ± 1.2                | 3.15 ± 0.94                |
| <u>intercalative binding</u> |                          |                                     |                            |                            |
| 3-aminoacridinium            | 10.17                    | -13.05                              | -13.7 ± 5                  | -2.2 ± 6                   |
| 7-aminoquinolinium           | 8.08                     | -11.02                              | -15.1 ± 4                  | -14 ± 13                   |

APPENDIX III  
COMPUTER PROGRAMS

These programs were designed for the calculations of apparent association constants and related data employing a Litton-Monroe model 326 Scientist computer. Terms not defined here are either defined in the text or are explained in the operator's manual for the computer. Brackets signify molar concentrations, unbracketed terms indicate moles. V denotes volume, subscripts o, t, and  $\infty$  refer to values before any DNA has been added, values at a given point in the titration, and values after excess DNA has been added, respectively.

#### Program I

This program has been designed to compute the following:  $[BH]$ ,  $[BHP]$ ,  $K_I$ ,  $\log([P_t/2] - 2[BHP])$ ,  $\log([BHP]/[BH])$ ,  $K_S$ , and  $\log([P_t/3] - [BHP])$ . For the program as written, the total initial volume must be 10.00 ml with a sample pathlength of 4.00 cm. Parameters which remain constant throughout a given titration are stored in registers 1, 2, 4, and 9 (their contents are listed in the table below). All data is entered as written, using scientific notation, and all results are displayed in their final form, that is, no correction factors are necessary. To run a program, enter total volume of DNA

solution ( $\mu\text{l}$ ), press START, enter total absorbance of the solution, press START. The program will be executed automatically, pausing for 3 to 6 seconds to display each of the above values in the sequence in which they are listed. To initiate another computational cycle, repeat the above procedure. At the completion of a computation, the registers will have stored within them the following information which may be recalled, if necessary.

| Register | Information                  | Register   | Information                              |
|----------|------------------------------|------------|------------------------------------------|
| 0        | $A_t$                        | 7          | $V_t$ in liters                          |
| 1        | $A_o$                        | 8          | Total l of DNA added up to point t       |
| 2        | $(A_o - A_\infty)^{-1} BH_o$ | 9          | $P_{t/2}$ (moles of DNA phosphate per l) |
| 3        | $[BHP]_t$                    | CHG<br>SGN | $K_I$                                    |
| 4        | $BH_o$                       | .          | $\log([P_{t/2}] - 2[BHP]_t)$             |
| 5        | $[BH]_t$                     |            |                                          |
| 6        | $[P_{t/3}]_t$                |            |                                          |

The individual steps of the program, along with their key-strokes and Munroe 326 code numbers are presented below.



| STEP | KEY<br>STROKES | CODE |
|------|----------------|------|
| 1    | ST             | 300  |
| 2    | 8              | 010  |
| 3    | STOP           | 033  |
| 4    | ST             | 300  |
| 5    | 0              | 000  |
| 6    | (              | 026  |
| 7    | RCL            | 310  |
| 8    | 8              | 010  |
| 9    | X              | 023  |
| 10   | EXP            | 014  |
| 11   | CHSN           | 013  |
| 12   | 6              | 006  |
| 13   | )              | 027  |
| 14   | +              | 021  |
| 15   | .              | 012  |
| 16   | 0              | 000  |
| 17   | 1              | 001  |
| 18   | =              | 020  |
| 19   | ST             | 300  |
| 20   | 7              | 007  |
| 21   | RCL            | 310  |
| 22   | 9              | 011  |
| 23   | X              | 023  |
| 24   | RCL            | 310  |
| 25   | 8              | 010  |
| 26   | ÷              | 024  |
| 27   | RCL            | 310  |
| 28   | 7              | 007  |
| 29   | =              | 020  |
| 30   | ST             | 300  |
| 31   | 6              | 006  |
| 32   | (              | 026  |
| 33   | RCL            | 310  |
| 34   | 1              | 001  |
| 35   | -              | 022  |
| 36   | RCL            | 310  |
| 37   | 0              | 000  |
| 38   | )              | 027  |
| 39   | X              | 023  |
| 40   | RCL            | 310  |
| 41   | 2              | 002  |
| 42   | =              | 020  |
| 43   | ST             | 300  |
| 44   | 3              | 003  |
| 45   | (              | 026  |
| 46   | RCL            | 310  |
| 47   | 4              | 004  |
| 48   | -              | 022  |
| 49   | RCL            | 310  |
| 50   | 3              | 003  |

| STEP | KEY<br>STROKES | CODE |
|------|----------------|------|
| 51   | )              | 027  |
| 52   | ÷              | 024  |
| 53   | RCL            | 310  |
| 54   | 7              | 007  |
| 55   | =              | 020  |
| 56   | ST             | 300  |
| 57   | 5              | 005  |
| 58   | fEXP           | 114  |
| 59   | RCL            | 310  |
| 60   | 3              | 003  |
| 61   | ÷              | 024  |
| 62   | RCL            | 310  |
| 63   | 7              | 007  |
| 64   | =              | 020  |
| 65   | ST             | 300  |
| 66   | 3              | 003  |
| 67   | fEXP           | 114  |
| 68   | RCL            | 310  |
| 69   | 3              | 003  |
| 70   | ÷              | 024  |
| 71   | (              | 026  |
| 72   | (              | 026  |
| 73   | RCL            | 310  |
| 74   | 6              | 006  |
| 75   | -              | 022  |
| 76   | (              | 026  |
| 77   | RCL            | 310  |
| 78   | 3              | 003  |
| 79   | X              | 023  |
| 80   | 2              | 002  |
| 81   | )              | 027  |
| 82   | )              | 027  |
| 83   | A <sup>x</sup> | 025  |
| 84   | 2              | 002  |
| 85   | X              | 023  |
| 86   | RCL            | 310  |
| 87   | 5              | 005  |
| 88   | )              | 027  |
| 89   | =              | 020  |
| 90   | ST             | 300  |
| 91   | CHSN           | 013  |
| 92   | fEXP           | 114  |
| 93   | (              | 026  |
| 94   | RCL            | 310  |
| 95   | 6              | 006  |
| 96   | -              | 022  |
| 97   | (              | 026  |
| 98   | RCL            | 310  |
| 99   | 3              | 003  |
| 100  | X              | 023  |

| STEP | KEY<br>STROKES | CODE | STEP | KEY<br>STROKES | CODE |
|------|----------------|------|------|----------------|------|
| 101  | 2              | 002  | 129  | 3              | 003  |
| 102  | )              | 027  | 130  | ÷              | 024  |
| 103  | )              | 027  | 131  | (              | 026  |
| 104  | ST             | 300  | 132  | RCL            | 310  |
| 105  | .              | 012  | 133  | 5              | 005  |
| 106  | LOG            | 061  | 134  | X              | 023  |
| 107  | fEXP           | 114  | 135  | (              | 026  |
| 108  | fEXP           | 114  | 136  | RCL            | 310  |
| 109  | (              | 026  | 137  | 6              | 006  |
| 110  | RCL            | 310  | 138  | -              | 022  |
| 111  | 3              | 003  | 139  | RCL            | 310  |
| 112  | ÷              | 024  | 140  | 003            | 003  |
| 113  | RCL            | 310  | 141  | )              | 027  |
| 114  | 5              | 005  | 142  | )              | 027  |
| 115  | )              | 027  | 143  | )              | 027  |
| 116  | LOG            | 061  | 144  | fEXP           | 114  |
| 117  | fEXP           | 114  | 145  | fEXP           | 114  |
| 118  | RCL            | 310  | 146  | (              | 026  |
| 119  | 6              | 006  | 147  | RCL            | 310  |
| 120  | ÷              | 024  | 148  | 6              | 006  |
| 121  | 1              | 001  | 149  | -              | 022  |
| 122  | .              | 012  | 150  | RCL            | 310  |
| 123  | 5              | 005  | 151  | 3              | 003  |
| 124  | =              | 020  | 152  | )              | 027  |
| 125  | ST             | 300  | 153  | LOG            | 061  |
| 126  | 6              | 006  | 154  | STOP           | 033  |
| 127  | (              | 026  | 155  | JUMP           | 350  |
| 128  | RCL            | 310  | 156  | START          | 033  |

### Program 2

This commercially available program (Litton-Monroe, Orange, N.J., number 9042W) solves the simultaneous equations (2-8), (2-9), and (2-10) for the concentrations of 7-aminoquinoline-containing species, [AHP], [AH], and [A]. Program execution is outlined in the operator's manual. The following table correlates the terminology of the program with the quantities used for this specific problem based on the general equations

$$A_1X + B_1Y + C_1Z = P$$

$$A_2X + B_2Y + C_3Z = Q$$

$$A_3X + B_3Y + C_3Z = R$$

| <u>Program designation</u> | <u>Variables or Coefficient</u>                                   |
|----------------------------|-------------------------------------------------------------------|
| X                          | [AHP]                                                             |
| Y                          | [AH]                                                              |
| Z                          | [A]                                                               |
| A <sub>1</sub>             | $\bar{\epsilon}_{\text{AHP}} (5332 \text{ M}^{-1}\text{cm}^{-1})$ |
| B <sub>1</sub>             | $\epsilon_{\text{AH}} (7940 \text{ M}^{-1}\text{cm}^{-1})$        |
| C <sub>1</sub>             | $\epsilon_{\text{A}} (347 \text{ M}^{-1}\text{cm}^{-1})$          |
| P                          | $A_t/4$                                                           |
| A <sub>2</sub>             | 1                                                                 |
| B <sub>2</sub>             | 1                                                                 |
| C <sub>2</sub>             | 1                                                                 |
| Q                          | $C_t = C_o/V_t$                                                   |
| A <sub>3</sub>             | 0                                                                 |
| B <sub>3</sub>             | $K_a (2.239 \times 10^{-7})$                                      |
| C <sub>3</sub>             | $-[H^+] (-1.259 \times 10^{-6})$                                  |
| R                          | 0                                                                 |

### Program 3

This program calculates association constants for the binding of 7-aminoquinolinium to DNA. The following are computed and displayed sequentially, as written:  $K_I$ ,  $\log([AHP]/[AH])$ ,  $\log([P_t/2] - 2[AHP])$ ,  $K_S$ , and  $\log([P_t/3] - [AHP])$ . A total

initial volume of 8.03 ml and a solution pathlength of 4.00 cm is required. Concentrations of AHP and AH are obtained from equations (2-8), (2-9), and (2-10), using Program 2. Before running a program (moles of DNA phosphate per  $\mu\text{l}$ )  $\cdot 1/2$  is loaded into register 9. To execute a program, enter  $\mu\text{l}$  of DNA solution, press START, enter  $[\text{AHP}] \times 10^5$ , press START, enter  $[\text{AH}] \times 10^5$ , press START. The concentrations of AHP and AH are converted to the proper orders of magnitude in the program. At the completion of a computation the registers will contain the following information for retrieval, if necessary.

| <u>Register</u> | <u>Information</u>                   | <u>Register</u> | <u>Information</u>                                                  |
|-----------------|--------------------------------------|-----------------|---------------------------------------------------------------------|
| 0               | $[\text{AHP}]_t$                     | 7               | $V_t$ in liters                                                     |
| 1               | $[\text{AH}]_t$                      | 8               | $\mu\text{l}$ of DNA                                                |
| 2               | $[\text{P}_{t/3}]_t$                 | 9               | $\text{P}_{t/2}$ (1/2 moles<br>DNA phosphate<br>per $\mu\text{l}$ ) |
| 3               | --                                   | .               | $K_s$                                                               |
| 4               | $\log([\text{AHP}]_t/[\text{AH}]_t)$ | CHG<br>SGN      | $K_I$                                                               |
| 5               | --                                   |                 |                                                                     |
| 6               | $[\text{P}_{t/2}]_t$                 |                 |                                                                     |

The individual steps of the program, along with their keystrokes and Munroe 326 code numbers are presented below.

| STEP | KEY<br>STROKES | CODE | STEP | KEY<br>STROKES | CODE |
|------|----------------|------|------|----------------|------|
| 1.   | ST             | 300  | 51   | CHSN           | 013  |
| 2    | 8              | 010  | 52   | 5              | 005  |
| 3    | STOP           | 033  | 53   | =              | 020  |
| 4    | ST             | 300  | 54   | ST             | 300  |
| 5    | 0              | 000  | 55   | 1              | 001  |
| 6    | STOP           | 033  | 56   | RCL            | 310  |
| 7    | ST             | 300  | 57   | 0              | 000  |
| 8    | 1              | 001  | 58   | ÷              | 024  |
| 9    | (              | 026  | 59   | (              | 026  |
| 10   | RCL            | 310  | 60   | (              | 026  |
| 11   | 8              | 010  | 61   | RCL            | 310  |
| 12   | X              | 023  | 62   | 6              | 006  |
| 13   | EXP            | 014  | 63   | -              | 022  |
| 14   | CHSN           | 013  | 64   | (              | 026  |
| 15   | 6              | 006  | 65   | RCL            | 310  |
| 16   | )              | 027  | 66   | 0              | 000  |
| 17   | +              | 021  | 67   | X              | 023  |
| 18   | .              | 012  | 68   | 2              | 002  |
| 19   | 0              | 000  | 69   | )              | 027  |
| 20   | 0              | 000  | 70   | )              | 027  |
| 21   | 8              | 010  | 71   | A <sup>x</sup> | 025  |
| 22   | =              | 020  | 72   | 2              | 002  |
| 23   | ST             | 300  | 73   | X              | 023  |
| 24   | 7              | 007  | 74   | RCL            | 310  |
| 25   | (              | 026  | 75   | 1              | 001  |
| 26   | RCL            | 310  | 76   | )              | 027  |
| 27   | 9              | 011  | 77   | =              | 020  |
| 28   | X              | 023  | 78   | ST             | 300  |
| 29   | RCL            | 310  | 79   | CHSN           | 013  |
| 30   | 8              | 010  | 80   | fEXP           | 114  |
| 31   | )              | 027  | 81   | (              | 026  |
| 32   | ÷              | 024  | 82   | RCL            | 310  |
| 33   | RCL            | 310  | 83   | 0              | 000  |
| 34   | 7              | 007  | 84   | ÷              | 024  |
| 35   | =              | 020  | 85   | RCL            | 310  |
| 36   | ST             | 300  | 86   | 1              | 001  |
| 37   | 6              | 006  | 87   | )              | 027  |
| 38   | RCL            | 310  | 88   | LOG            | 061  |
| 39   | 0              | 000  | 89   | ST             | 300  |
| 40   | X              | 023  | 90   | 4              | 004  |
| 41   | EXP            | 014  | 91   | fEXP           | 114  |
| 42   | CHSN           | 013  | 92   | (              | 026  |
| 43   | 5              | 005  | 93   | RCL            | 310  |
| 44   | =              | 020  | 94   | 6              | 006  |
| 45   | ST             | 300  | 95   | -              | 022  |
| 46   | 0              | 000  | 96   | (              | 026  |
| 47   | RCL            | 310  | 97   | RCL            | 310  |
| 48   | 1              | 001  | 98   | 0              | 000  |
| 49   | X              | 023  | 99   | X              | 023  |
| 50   | EXP            | 014  | 100  | 2              | 002  |

| STEP | KEY<br>STROKES | CODE | STEP | KEY<br>STROKES | CODE |
|------|----------------|------|------|----------------|------|
| 101  | )              | 027  | 124  | RCL            | 310  |
| 102  | )              | 027  | 125  | 0              | 000  |
| 103  | LOG            | 061  | 126  | )              | 027  |
| 104  | STOP           | 033  | 127  | X              | 023  |
| 105  | RCL            | 310  | 128  | RCL            | 310  |
| 106  | 6              | 006  | 129  | 1              | 001  |
| 107  | X              | 023  | 130  | )              | 027  |
| 108  | .              | 012  | 131  | =              | 020  |
| 109  | 6              | 006  | 132  | ST             | 300  |
| 110  | 6              | 006  | 133  | .              | 012  |
| 111  | 6              | 006  | 134  | fEXP           | 114  |
| 112  | 6              | 006  | 135  | (              | 026  |
| 113  | =              | 020  | 136  | RCL            | 310  |
| 114  | ST             | 300  | 137  | 2              | 002  |
| 115  | 2              | 002  | 138  | -              | 022  |
| 116  | RCL            | 310  | 139  | RCL            | 310  |
| 117  | 0              | 000  | 140  | 0              | 000  |
| 118  | %              | 024  | 141  | )              | 027  |
| 119  | (              | 026  | 142  | LOG            | 061  |
| 120  | (              | 026  | 143  | STOP           | 033  |
| 121  | RCL            | 310  | 144  | JUMP           | 350  |
| 122  | 2              | 022  | 145  | START          | 033  |
| 123  | -              | 022  |      |                |      |

#### Program 4

This program calculates the activity coefficients of species at a given ionic strength based on equation (1-30). The coefficients may be stored for subsequent determination of  $K'$  from  $K$  [equation (2-17)]. The program is designed for aqueous systems at 25.0°C for which  $B$  is equal to  $3.3 \times 10^7$ . To execute a program, enter  $I$ , press ST 7, enter  $d$  for the  $i^{\text{th}}$  species, press ST 8, enter  $Z_i$ , press ST 6, press JUMP, CHG SGN, START. The value of  $\alpha_i$  will be displayed. Store the values of  $\alpha_{\text{BHP}}$ ,  $\alpha_{\text{BH}}$ , and  $\alpha_{\text{p}}$  or the respective values for AHP, AH, and A in registers 4, 2, and 5, respectively. Upon storing these values, the correction term for

intercalative binding [ $n = 2$  of equation (2-17)] may be computed by pressing JUMP, 9, START]. For the surface binding correction factor press JUMP, ., START.

At the completion of a series of computations, the registers will contain the following information which may be retrieved, if necessary.

| Register | Information                                                              | Register   | Information                  |
|----------|--------------------------------------------------------------------------|------------|------------------------------|
| 0        | $\alpha_{\text{BHP}} / (\alpha_{\text{BH}} \cdot \alpha_{\text{P}_t})$   | 7          | I                            |
| 1        | $\alpha_{\text{BHP}} / (\alpha_{\text{BH}} \cdot \alpha_{\text{P}_t}^2)$ | 8          | $d_i$ (the last one entered) |
| 2        | $\alpha_{\text{BH}}$                                                     | 9          | --                           |
| 3        | --                                                                       | .          | --                           |
| 4        | $\alpha_{\text{BHP}}$                                                    | CHG<br>SGN | ---                          |
| 6        | $z_i$ (the last one entered)                                             |            |                              |

The individual steps of the program, along with their keystrokes and Munroe 326 code numbers are presented below.

| STEP | KEY<br>STROKES | CODE | STEP | KEY<br>STROKES | CODE |
|------|----------------|------|------|----------------|------|
| 1    | f.LABEL        | 213  | 11   | 7              | 007  |
| 2    | (              | 026  | 12   | A <sup>x</sup> | 025  |
| 3    | (              | 026  | 13   | .              | 012  |
| 4    | RCL            | 310  | 14   | 5              | 005  |
| 5    | 6              | 006  | 15   | X              | 023  |
| 6    | A <sup>x</sup> | 025  | 16   | .              | 012  |
| 7    | 2              | 002  | 17   | 5              | 005  |
| 8    | X              | 023  | 18   | 0              | 000  |
| 9    | (              | 026  | 19   | 9              | 011  |
| 10   | RCL            | 310  | 20   | )              | 027  |

| STEP | KEY<br>STROKES | CODE | STEP | KEY<br>STROKES | CODE |
|------|----------------|------|------|----------------|------|
| 21   | )              | 027  | 51   | RCL            | 310  |
| 22   | ÷              | 024  | 52   | 4              | 004  |
| 23   | (              | 026  | 53   | ÷              | 024  |
| 24   | (              | 026  | 54   | (              | 026  |
| 25   | RCL            | 310  | 55   | RCL            | 310  |
| 26   | 7              | 007  | 56   | 5              | 005  |
| 27   | A <sup>x</sup> | 025  | 57   | X              | 023  |
| 28   | .              | 012  | 58   | RCL            | 310  |
| 29   | 5              | 005  | 59   | 2              | 002  |
| 30   | X              | 023  | 60   | )              | 027  |
| 31   | RCL            | 310  | 61   | )              | 027  |
| 32   | 8              | 010  | 62   | ST             | 300  |
| 33   | X              | 023  | 63   | 0              | 000  |
| 34   | 3              | 003  | 64   | STOP           | 033  |
| 35   | .              | 012  | 65   | JUMP           | 350  |
| 36   | 3              | 003  | 66   | .              | 012  |
| 37   | EXP            | 014  | 67   | fLABEL9        | 211  |
| 38   | 7              | 007  | 68   | (              | 026  |
| 39   | )              | 027  | 69   | RCL            | 310  |
| 40   | +              | 021  | 70   | 0              | 000  |
| 41   | 1              | 001  | 71   | ÷              | 024  |
| 42   | )              | 027  | 72   | RCL            | 310  |
| 43   | )              | 027  | 73   | 5              | 005  |
| 44   | CHSN           | 013  | 74   | )              | 027  |
| 45   | ALOG           | 161  | 75   | ST             | 300  |
| 46   | STOP           | 033  | 76   | 1              | 001  |
| 47   | JUMP           | 350  | 77   | STOP           | 033  |
| 48   | CHSN           | 013  | 78   | JUMP           | 350  |
| 49   | fLABEL.        | 212  | 79   | 9              | 011  |
| 50   | (              | 026  |      |                |      |

#### Program 5

This program calculates the ionic strength of a solution resulting from the addition of a given volume of DNA to 10.00 ml of solution having a known initial ionic strength. The contribution of background electrolyte in the DNA solution is included. An initial and final volume of DNA solution may be entered to obtain an averaged ionic strength over a range. Calculations for solutions containing monovalent or divalent cations may be made depending on the



constants used for steps 51 and 67 of the program. Before running the program, the normality of the 10.00 ml solution containing the BH or AH is stored in register 0 and  $1/2$  (moles of DNA phosphate + equivalents of supporting electrolyte in the DNA solution) per  $\mu\text{l}$  is stored in register 2. To execute a program, enter  $V_1$  of DNA solution ( $\mu\text{l}$ ), press START, enter  $V_2$  of DNA solution ( $\mu\text{l}$ ), press START. The averaged ionic strength will be displayed. If the total final ionic strength of a solution containing a given, single, volume of DNA is desired, execute the program by entering that value ( $\mu\text{l}$ ) and pressing START, START. At the completion of a calculation the registers will contain the following information which may be retrieved, if necessary.

| Register | Information                                                                                          |
|----------|------------------------------------------------------------------------------------------------------|
| 0        | Normality, N, of the 10.00 ml solution before any DNA has been added.                                |
| 1        | Average volume of DNA added ( $\mu\text{l}$ ) = $(V_1\text{DNA} + V_2\text{DNA})/2$ .                |
| 2        | $1/2$ (moles of DNA phosphate + equivalents of supporting electrolyte in the DNA solution).          |
| 3        | Total final average volume of solution = $0.01000 + \text{average volume of DNA added, in liters}$ . |
| 4        | Total final volume of solution $\times 10^2$ .                                                       |
| 5        | Ionic strength, I, of the final solution if register 0 contains 0.                                   |
| 6        | I of the final solution if register 2 contains 0 (dilution effect only).                             |

| Register   | Information                                                        |
|------------|--------------------------------------------------------------------|
| 7          | $V_1$ DNA ( $\mu$ l).                                              |
| 8          | $V_2$ DNA ( $\mu$ l).                                              |
| 9          | I of final solution (averaged volume and averaged ionic strength). |
| .          | --                                                                 |
| CHG<br>SGN | --                                                                 |

The individual steps of the program, along with their keystrokes and Munroe 326 code numbers are presented below.

| STEP | KEY<br>STROKES | CODE | STEP | KEY<br>STROKES | CODE |
|------|----------------|------|------|----------------|------|
| 1    | ST             | 300  | 31   | 2              | 002  |
| 2    | 7              | 007  | 32   | )              | 027  |
| 3    | STOP           | 033  | 33   | ST             | 300  |
| 4    | ST             | 300  | 34   | 3              | 003  |
| 5    | 8              | 010  | 35   | (              | 026  |
| 6    | (              | 026  | 36   | RCL            | 310  |
| 7    | (              | 026  | 37   | 3              | 003  |
| 8    | RCL            | 310  | 38   | X              | 023  |
| 9    | 7              | 007  | 39   | EXP            | 014  |
| 10   | +              | 021  | 40   | 2              | 002  |
| 11   | RCL            | 310  | 41   | )              | 027  |
| 12   | 8              | 010  | 42   | ST             | 300  |
| 13   | )              | 027  | 43   | 4              | 004  |
| 14   | $\div$         | 024  | 44   | (              | 026  |
| 15   | 2              | 002  | 45   | (              | 026  |
| 16   | )              | 027  | 46   | RCL            | 310  |
| 17   | ST             | 300  | 47   | 1              | 001  |
| 18   | 1              | 001  | 48   | X              | 023  |
| 19   | (              | 026  | 49   | RCL            | 310  |
| 20   | (              | 026  | 50   | 2              | 002  |
| 21   | RCL            | 310  | 51   | X              | 023  |
| 22   | 1              | 001  | 52   | 3*             | 003* |
| 23   | X              | 023  | 53   | )              | 027  |
| 24   | EXP            | 014  | 54   | $\div$         | 024  |
| 25   | CHSN           | 013  | 55   | RCL            | 310  |
| 26   | 6              | 006  | 56   | 3              | 003  |
| 27   | )              | 027  | 57   | )              | 027  |
| 28   | +              | 021  | 58   | ST             | 300  |
| 29   | EXP            | 014  | 59   | 5              | 005  |
| 30   | CHSN           | 013  | 60   | (              | 026  |

| STEP | KEY<br>STROKES | CODE  |
|------|----------------|-------|
| 61   | (              | 026   |
| 62   | RCL            | 310   |
| 63   | 0              | 000   |
| 64   | X              | 023   |
| 65   | 1              | 001   |
| 66   | .              | 012   |
| 67   | 5**            | 005** |
| 68   | )              | 027   |
| 69   | ÷              | 024   |
| 70   | RCL            | 310   |
| 71   | 4              | 004   |
| 72   | )              | 027   |
| 73   | ST             | 300   |
| 74   | 6              | 006   |
| 75   | (              | 026   |
| 76   | RCL            | 310   |
| 77   | 5              | 005   |
| 78   | +              | 021   |

| STEP | KEY<br>STROKES | CODE |
|------|----------------|------|
| 79   | RCL            | 310  |
| 80   | 6              | 006  |
| 81   | )              | 027  |
| 82   | ST             | 300  |
| 83   | 9              | 011  |
| 84   | STOP           | 033  |
| 85   | JUMP           | 350  |
| 86   | START          | 033  |

\* 51 3 003 for  $M^{2+}$   
 51 2 002 for  $M^{+1}$

\*\* 67 5 005 for  $M^{2+}$   
 67 0 000 for  $M^{1+}$

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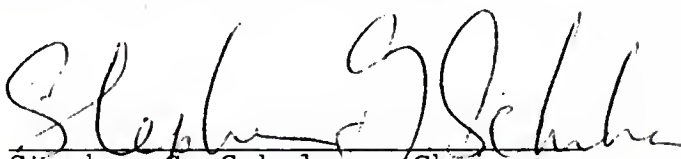
## BIOGRAPHICAL SKETCH

Peter F. Eisenhardt was born on March 18, 1945, in Cortland, New York. He graduated from Dryden Central High School in June, 1963. In September, 1963, he entered SUNY at Cortland and graduated in June, 1967, with a Bachelor of Science degree in chemistry. He entered the Department of Chemistry at the University of Florida in the Fall of 1968 and received a Master of Science degree in inorganic chemistry in June, 1971. For the next three years he was employed at the College of Pharmacy, University of Florida where he developed analytical methods for an ophthalmic ointment development program, before beginning studies toward his doctorate in September, 1974. He is a member of the American Pharmaceutical Association, Academy of Pharmaceutical Sciences, American Chemical Society, Florida Section of the American Chemical Society, Alpha Chi Sigma Chemistry Fraternity, and Delta Kappa Pi.

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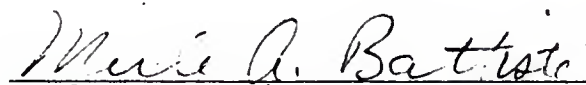
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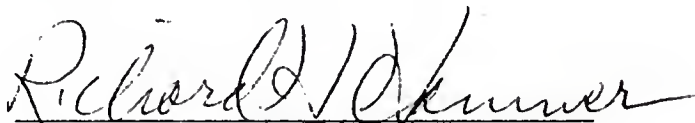
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